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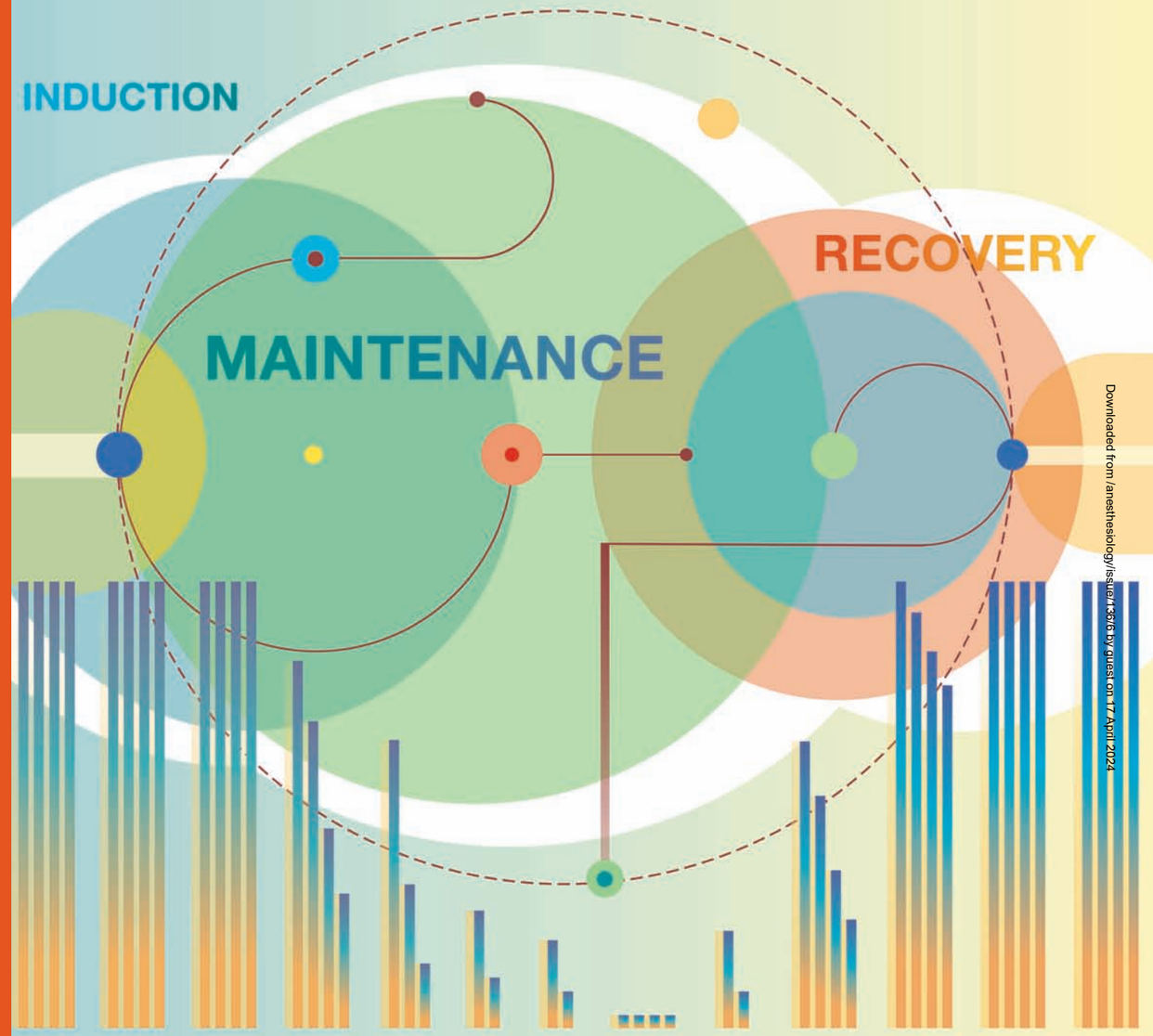

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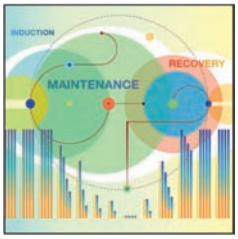
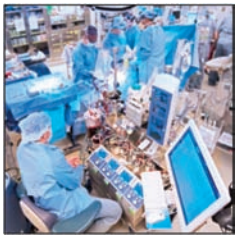


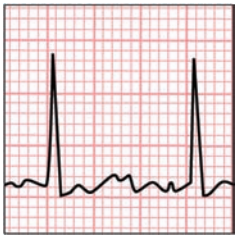
901 Quantitative Neuromuscular Monitoring in Clinical Practice: A Professional Practice Change Initiative

Quantitative (train-of-four ratio) monitoring is the gold standard for assessing recovery from neuromuscular block. Residual neuromuscular block, defined as a train-of-four ratio of less than 0.9, is commonly observed in patients given nondepolarizing neuromuscular blocking drugs perioperatively. Inadequate reversal of residual neuromuscular block is associated with postoperative morbidity and mortality. Despite guidelines from several professional societies advocating quantitative neuromuscular monitoring for neuromuscular blocking drug management, it is infrequently used. A departmental professional practice change initiative was initiated with the goal of documenting a train-of-four ratio greater than or equal to 0.9 for all patients given a nondepolarizing neuromuscular blocking drug. This retrospective assessment of implementation of train-of-four ratio greater than or equal to 0.9 documentation before extubation found it improved from 2 (1%) of 172 cases in November 2016, which was preimplementation, to 250 (93%) of 269 cases in December 2020, which was postimplementation. Attaining this endpoint required not only placing a quantitative monitor in each anesthetizing location but also ongoing educational efforts and follow-up. *See the accompanying Editorial on* ***page 875****. (Summary: M. J. Avram. Image: A. Johnson, Vivo Visuals Studio.)*



927 Intraoperative Hypotension and Acute Kidney Injury, Stroke, and Mortality during and outside Cardiopulmonary Bypass: A Retrospective Observational Cohort Study

Intraoperative hypotension is associated with major adverse postoperative events in patients having noncardiac surgery. The hypothesis that intraoperative hypotension duration throughout cardiac surgery or when separated into hypotension during and outside cardiopulmonary bypass (CPB) may be associated with major adverse postoperative events was tested in a retrospective study. The composite primary outcome of postoperative acute kidney injury, stroke, and mortality occurred in 256 (5%) of 4,984 patients. The mean ± SD duration of mean arterial pressure (MAP) less than 65 mmHg was 143 ± 75 min in patients with the composite primary outcome and 104 ± 48 min in those without it. The area under the curve–MAP less than 65 mmHg, a measure of the severity of intraoperative hypotension, was 1,528 ± 1,134 mmHg · min in patients with the composite outcome and 1,070 ± 656 mmHg · min in the others. When compared with more than 80% hypotension duration occurring during CPB, less than 60% of hypotension occurring during CPB was associated with the primary composite outcome (odds ratio, 1.67; 95% CI, 1.10 to 2.60). *(Summary: M. J. Avram. Image: J. P. Rathmell.)*



916 Amiodarone with or without *N*-Acetylcysteine for the Prevention of Atrial Fibrillation after Thoracic Surgery: A Double-blind, Randomized Trial

Transient atrial fibrillation after noncardiac thoracic or general surgery may be a sentinel event that can be used to identify patients at risk of developing subsequent atrial fibrillation. More than 15% of patients at high risk of postoperative atrial fibrillation will experience it after thoracic surgery when the multichannel antiarrhythmic drug amiodarone is used for prevention. The hypothesis that the addition of *N*-acetylcysteine, an antioxidant anti-inflammatory agent, to amiodarone would reduce the incidence of postoperative atrial fibrillation compared with amiodarone alone was tested in a randomized, double-blind, placebo-controlled trial of 154 patients at high risk of postoperative atrial fibrillation scheduled to undergo major thoracic surgery. Interim analysis found the primary outcome, new-onset sustained atrial fibrillation for more than 30 s detected by telemetry (first 72 h) or symptomatic atrial fibrillation requiring intervention and confirmed by electrocardiography within 7 days of surgery, occurred in 15 (19%) of 78 patients in the *N*-acetylcysteine group and 13 (17%) of 76 patients in the placebo group. The trial was terminated due to futility. *See the accompanying Editorial on* ***page 877****. (Summary: M. J. Avram. Image: J. P. Rathmell.)*

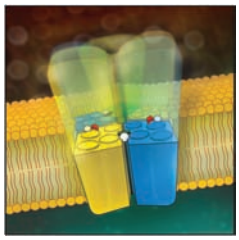
970 Basal Infusion *versus* Automated Boluses and a Delayed Start Timer for "Continuous" Sciatic Nerve Blocks after Ambulatory Foot and Ankle Surgery: A Randomized Clinical Trial

Analgesia after foot and ankle surgery is often provided by a popliteal–sciatic nerve block produced by a continuous basal local anesthetic infusion and patient-controlled bolus doses. The hypotheses that, compared to a continuous basal ropivacaine infusion initiated before discharge after foot and ankle surgery, perineural ropivacaine administered with automated boluses at a lower dose and a 5-h delay after discharge would provide at least noninferior analgesia while both techniques are functioning and result in a longer duration of administration were tested in a randomized controlled trial of 70 patients. The day after surgery, the median [interquartile range] pain score of participants receiving automated boluses was 0.0 [0.0 to 3.0] while that of the continuous infusion group was 3.0 [1.8 to 4.8]. The odds of worse average pain on day 1 with continuous basal infusion, adjusting for body mass index, was 3.1 (95% CI, 1.2 to 7.8). Local anesthetic reservoir exhaustion occurred after a median [interquartile range] of 119 h [109 to 125 h] in patients with automated boluses and 74 h [57 to 80 h] in the continuous infusion group. *See the accompanying Editorial on* ***page 883****. (Summary: M. J. Avram. Image: J. P. Rathmell/A. Johnson, Vivo Visuals Studio.)*

DOI: 10.1097/ALN.0000000000004244

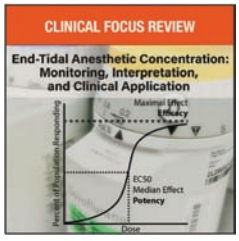
940 Referral Indications for Malignant Hyperthermia Susceptibility Diagnostics in Patients without Adverse Anesthetic Events in the Era of Next-generation Sequencing

Most cases of malignant hyperthermia susceptibility are associated with variants in the gene encoding the skeletal muscle ryanodine receptor 1, *RYR1*. Next-generation sequencing has resulted in a rapid increase in the identification of both the number of patients with an *RYR* variant and the number of newly identified *RYR1* variants. The hypothesis that there is an increased referral to malignant hyperthermia units of patients without a personal or family history of adverse anesthetic events suspected to be malignant hyperthermia was tested in a retrospective multicenter cohort study. The proportion of patients referred without a personal or family history of adverse anesthetic events suspected to be malignant hyperthermia increased from 28% (61 of 215) between 2010 and 2014 to 44% (133 of 305) between 2015 and 2019. Patients with a personal or family history of adverse anesthetic events suspected to be malignant hyperthermia were more frequently diagnosed as malignant hyperthermia susceptible (133 of 220; 60%) than those without (47 of 120; 39%). *(Summary: M. J. Avram. Image: J. P. Rathmell.)*



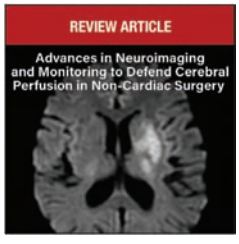
954 Midazolam at Low Nanomolar Concentrations Affects Long-term Potentiation and Synaptic Transmission Predominantly *via* the α_1–γ-Aminobutyric Acid Type A Receptor Subunit in Mice

Midazolam at low nanomolar concentrations causes moderate sedation and anterograde amnesia in humans. Consistent with its amnestic properties, midazolam blocks hippocampal long-term potentiation, a cellular correlate for learning and memory. The sedative properties of benzodiazepines are mediated by α_1-containing γ-aminobutyric acid type A ($GABA_A$) receptors expressed in forebrain glutamatergic neurons. The aims of this study were to identify the $GABA_A$ receptor subtypes targeted by midazolam responsible for affecting long-term potentiation and synaptic inhibition in neocortical neurons. Mouse lines carrying knock-in mutations in the α-subunit of the $GABA_A$ receptor, which cause a dramatic decrease in benzodiazepine binding, were used to determine the effect of midazolam on hippocampal neurons in electrophysiologic studies of acutely prepared brain slices. Midazolam at 10 nM completely blocked long-term potentiation. It was effective in blocking long-term potentiation in slices derived from $\alpha_{2/3/5}$–triple–knock-in mice and it failed to block long-term potentiation in slices derived from α_1–single–knock-in mice, leading to the conclusion that midazolam blocked hippocampal long-term potentiation predominantly via α_1-$GABA_A$ receptors. *See the accompanying Editorial on* ***page 880****. (Summary: M. J. Avram. Image: A. Johnson, Vivo Visuals Studio.)*



985 End-tidal Anesthetic Concentration: Monitoring, Interpretation, and Clinical Application (Clinical Focus Review)

This Clinical Focus Review begins by describing the development of the concept of minimal alveolar concentration (MAC), the volatile anesthetic steady-state end-tidal partial pressure that results in a 50% probability of subject immobility after application of a noxious stimulus, and the semantic issues that have plagued the term. The current understanding of general anesthesia is then presented as a drug-induced reversible state of unconsciousness while providing immobility with blunting of excessive autonomic responses to noxious stimuli. This is followed by a discussion of the relationships between the steady-state fraction of MAC and each of these clinical effects and the effects of opioids on these relationships. Hysteresis, the delay between changes in the fraction of end-tidal anesthetic concentration and changes in the partial pressure of anesthetic in the brain during non–steady-state conditions, and hysteresis-corrected fraction of MAC are then considered. The review concludes with a presentation of a pragmatic approach to achieving the anesthetic state based on the MAC and the fraction of MAC concepts and a discussion of the future of MAC. *See the accompanying Editorial on* ***page 885****. (Summary: M. J. Avram. Image: J. P. Rathmell.)*



1015 Advances in Neuroimaging and Monitoring to Defend Cerebral Perfusion in Noncardiac Surgery (Review Article)

The brain is susceptible to perioperative injury from hypoperfusion and oxygen supply–demand mismatch. This review focuses on emerging techniques that have the capacity to perform multisite measurement/imaging of biomarkers of neurologic injury, potentially enabling early detection of organ hypoperfusion and avoidance of tissue oxygen supply–demand mismatch: optical, ultrasonographic, and magnetic resonance techniques. Optical techniques, such as near-infrared spectroscopy, are able to measure a range of properties of superficial cortical tissue continuously and noninvasively, thereby measuring surrogates of cerebral blood flow, cerebral oxygenation, and cellular oxygenation and/or metabolism. Transcranial Doppler ultrasound is a well-established technique for evaluating cerebral perfusion intraoperatively. Magnetic resonance imaging can be used to diagnose perioperative stroke in the early stages and, due to its exquisite spatial resolution, is excellent for assessing regional blood flow in the brain. Although novel neuroimaging modalities may revolutionize perioperative care, few studies have assessed application of these methods in the perioperative setting. *(Summary: M. J. Avram. Image: J. P. Rathmell.)*

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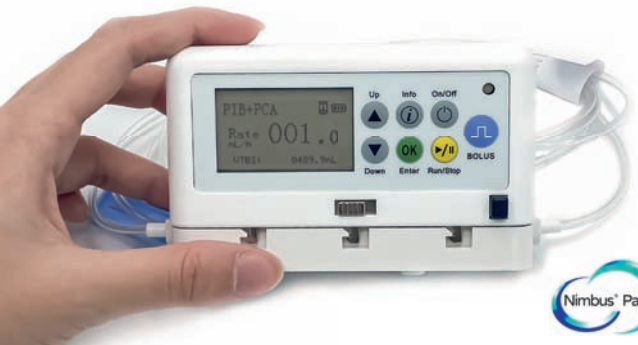

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[1] Finneran J, et al, Replacing the Basal Infusion with Automated Boluses and a Delayed Start Timer for "Continuous" Sciatic Nerve Blocks. ASRA 2022 Abstract 3104 https://www.asra.com/docs/default-source/events-education-documents/raapm22/abstract-3104.pdf?sfvrsn=addeeb14_2.

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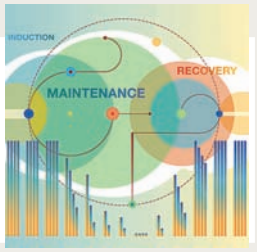

ON THE COVER: Residual neuromuscular blockade can be avoided with quantitative neuromuscular monitoring. In this issue of ANESTHESIOLOGY, Weigel *et al.* detail a professional practice initiative they conducted to attain documented train-of-four ratios greater than or equal to 0.90 in all patients to improve patient outcomes through reducing residual paralysis. In an accompanying editorial, Lane-Fall tells us how this new article demonstrates much about what anesthesiology has to learn from implementation science and quality improvement. Cover Illustration: A. Johnson, Vivo Visuals Studio.

- Weigel *et al.*: Quantitative Neuromuscular Monitoring in Clinical Practice: A Professional Practice Change Initiative, p. 901
- Lane-Fall: What Anesthesiology Has to Learn from Implementation Science and Quality Improvement, p. 875

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Read the article by Finneran *et al.* entitled "Basal Infusion *versus* Automated Boluses and a Delayed Start Timer for "Continuous" Sciatic Nerve Blocks after Ambulatory Foot and Ankle Surgery: A Randomized Clinical Trial" on page 970.

Learning Objectives

After successfully completing this activity, the learner will be able to counsel patients on the potential effectiveness of ambulatory regional anesthetic techniques for postoperative pain management (1,2,3), consider the likelihood of breakthrough pain based on regional management (1,2), and anticipate opioid requirements based on the administration technique selected (3).

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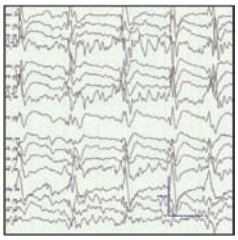
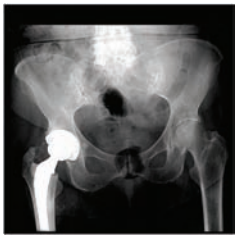


Treating rhythmic and periodic EEG patterns in comatose survivors of cardiac arrest. N Engl J Med 2022; 386:724–34. PMID: 35196426.

Pharmacologic suppression of rhythmic and periodic electroencephalographic (EEG) patterns in comatose survivors of cardiac arrest remains controversial. This study reports a randomized trial (11 European intensive care units [ICUs]) of rhythm and periodic EEG pattern suppression (on continuous EEG monitoring) in this setting. A stepwise strategy of antiseizure suppressive medications for 48 h (antiseizure drugs plus sedation within 3 h of detection) plus standard care or standard care alone (targeted temperature management) were compared. The primary outcome was neurologic outcome dichotomized as either good or poor outcome using the Cerebral Performance Category scale at 3 months postrandomization. One hundred seventy-two patients were randomized. Abnormal EEG activity were detected at a median of 35 h after arrest. Complete suppression occurred in 56% of the treated group *versus* 2% of control. At 3 months, no differences were noted: 90% of the antiseizure-treatment group *versus* 92% of the control group had a poor outcome (difference, 2 percentage points; 95% CI, –7 to 11; $P = 0.68$). Mortality at 3 months was 80% in the treatment group *versus* 82% in the control group. The mean length of stay in the ICU and duration of mechanical ventilation were slightly longer in the treatment group than in the control group. *(Article Selection: Martin J. London, M.D. Image: Jong Woo Lee, M.D., Ph.D.)*

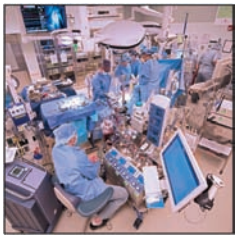
Take home message: In comatose survivors of cardiac arrest, the incidence of a poor neurologic outcome at 3 months did not differ significantly between a strategy of suppressing rhythmic and periodic EEG activity with the use of antiseizure medication for at least 48 h plus standard care and standard care alone.



Cemented or uncemented hemiarthroplasty for intracapsular hip fracture. N Engl J Med 2022; 386:521–30. PMID: 35139272.

Given limited available postoperative quality-of-life data, equipoise exists regarding the need for bone cement in hip fractures treated with hemiarthroplasty. This study reports a randomized controlled trial of cemented *versus* uncemented hemiarthroplasty in patients with an intracapsular fracture aged 60 yr or older at 14 UK centers. Perioperative process variables were all dictated by local center policies. The primary outcome (health-related quality of life) was assessed with utility scores on the EuroQol Group 5-Dimension (EQ-5D) questionnaire *via* telephone 4 months after randomization (range of scores, –0.594 to 1, with higher scores reflecting better quality of life; minimal clinically important difference, 0.050 to 0.075). Primary outcome data were available for 72% of randomized patients (610 cemented, 615 uncemented). A modest but statistically significant improvement in quality of life was noted in the cemented group: mean EQ-5D utility score 0.371 *versus* 0.315 (adjusted difference, 0.055; 95% CI, 0.009 to 0.101; $P = 0.02$). Periprosthetic fracture was lower in the cemented group (0.5% *vs.* 2.1%; odds ratio [uncemented *vs.* cemented], 4.37; 95% CI, 1.19 to 24.00). Other complications were similar in the two groups. *(Article Selection: Martin J. London, M.D. Image: J. P. Rathmell.)*

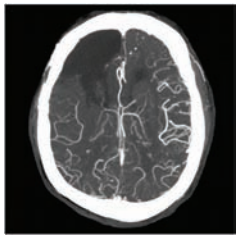
Take home message: In a large randomized trial, quality of life was better and periprosthetic fracture was lower in patients with cemented hemiarthroplasty after intracapsular hip fracture.



Ten-year outcomes of off-pump vs on-pump coronary artery bypass grafting in the Department of Veterans Affairs: A randomized clinical trial. JAMA Surg 2022; 157:303–10. PMID: 35171210.

From February 2002 to May 2007, a randomized controlled trial (Randomized On/Off Bypass trial [ROOBY]) was performed including more than 2,000 veterans. Patients were randomized to on-pump or off-pump coronary artery bypass grafting (CABG) procedures in 18 Veterans Affairs (VA) medical centers. Results have previously been published at 1 and 5 yr of follow-up. The 10-yr co–primary endpoints included all-cause mortality or a composite of subsequent revascularization (percutaneous coronary intervention [PCI] or repeat CABG) or death, assessed dichotomously and as time-to-events. Secondary outcomes included PCIs, repeat CABG, cardiac symptoms, and estimated costs. Outcome information was collected *via* electronic medical records in combination with VA and non-VA databases. Intention-to treat analysis revealed no difference in 10-yr mortality: 31% on-pump in 1,099 patients (mean age, 62.5 yr) *versus* 34% off-pump in 1,104 patients (mean age, 62.3 yr) (relative risk, 1.05; 95% CI, 0.99 to 1.11; $P = 0.12$). The median time to reach the composite endpoint in the on-pump was significantly shorter in the off-pump group, 4.6 yr (interquartile range, 1.4 to 7.5 yr) *versus* 5.0 yr (interquartile range, 1.8 to 7.9 yr) on-pump; $P = 0.03$. All other endpoints were comparable between the two groups. Results were also confirmed in sensitivity analyses. *(Article Selection: Beatrice Beck-Schimmer, M.D. Image: J. P. Rathmell.)*

Take home message: In this large, selected cohort of veteran patients enrolled in the landmark ROOBY trial, no long-term advantages were found for off-pump CABG.



Endovascular therapy for acute stroke with a large ischemic region. N Engl J Med 2022; 386:1303–13. PMID: 35138767.

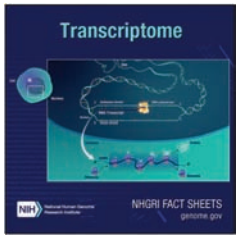
The role of endovascular therapy *versus* medical care alone for large acute ischemic strokes (which is generally avoided due to risk of postreperfusion bleeding) has not been well studied. This is a multicenter, randomized trial in Japan of patients with large strokes on imaging, as indicated by an Alberta Stroke Program Early Computed Tomographic Score (ASPECTS) value of 3 to 5 (scale, 0 to 10; lower values indicate larger infarction). Patients were randomized to receive endovascular therapy with medical care or medical care alone within 6 h after symptom onset or within 24 h if there was no early change on imaging. One hundred one patients were randomized to endovascular therapy and 102 to the medical care group; 27% in each group received alteplase (0.6 mg/kg). The primary outcome was the percentage of patients with a modified Rankin scale score of 0 to 3 (scale, 0 to 6; higher scores indicate greater disability) at 90 days. The primary outcome was better in the endovascular therapy group relative to medical therapy alone (31% *vs.* 13%; relative risk, 2.43; 95% CI, 1.35 to 4.37; $P = 0.002$). Any intracranial hemorrhage occurred in 58% and 31%, respectively ($P < 0.001$). *(Article Selection: David Faraoni, M.D., Ph.D. Image: J. P. Rathmell.)*

Take home message: Patients with large cerebral infarctions had better functional outcomes with endovascular therapy compared to medical care alone, albeit with more intracranial hemorrhages.

Regular acetaminophen use and blood pressure in people with hypertension: The PATH-BP Trial. Circulation 2022; 145:416–23. PMID: 35130054.

Observational studies suggest that acetaminophen may increase blood pressure, but clinical trials are lacking. This single-center, double-blind, randomized, investigator-initiated crossover study enrolled 110 individuals with a history of hypertension to receive 4 g acetaminophen daily in divided doses or matched placebo for 2 weeks. Patients crossed over to the alternate treatment after a 2-week washout period. At the beginning and end of each treatment period, 24-hr ambulatory blood pressure readings were measured. The primary outcome was the change in mean daytime systolic blood pressure from baseline to end of treatment between the placebo and acetaminophen arms. One-hundred three patients completed both arms of the study. Those taking acetaminophen, compared with placebo, had significantly greater mean daytime systolic blood pressure (133 ± 10 to 136 ± 10 mmHg [acetaminophen] *vs.* 134 ± 10 to 132 ± 10 mmHg [placebo]; $P < 0.0001$) with a placebo-corrected rise of 4.7 mmHg (95% CI, 2.9 to 6.6). Mean daytime diastolic blood pressure was also greater in patients taking acetaminophen (81 ± 8 to 82 ± 8 mmHg in the acetaminophen group *vs.* 82 ± 8 to 81 ± 8 mmHg in the placebo patients; $P = 0.005$) with a placebo-corrected rise of 1.6 mmHg (95% CI, 0.5 to 2.7). Similar findings were seen for 24-hr ambulatory and clinic blood pressure readings. *(Article Selection: BobbieJean Sweitzer, M.D. Image: J. P. Rathmell.)*

Take home message: Individuals with hypertension taking 4 g acetaminophen daily had greater systolic blood pressure by approximately 5 mmHg when compared with placebo, raising concerns about safety in those with cardiovascular risk factors.



System-wide transcriptome damage and tissue identity loss in COVID-19 patients. Cell Rep Med 2022; 3:100522. PMID: 35233546.

The molecular mechanisms underpinning the clinical presentation of patients with SARS-CoV-2 infection are poorly characterized. Body-wide and tissue-compartment-specific transcriptional profiling, combined with imaging, were applied to nasopharyngeal swabs and autopsy tissue from 39 patients who died from SARS-CoV-2 infection and compared with 22 healthy organ donor samples and non–COVID-19 acute lung injury samples. Duration of illness was inversely proportional to viral load. There was loss of tissue type identity. The COVID-19 samples were marked by a ubiquitous increase in fibroblasts and immune cells but a loss of organ-specific major cell types, such as alveolar epithelial cells in the lung and cardiomyocytes in the heart. Interferon- and cytokine-related pathways increased in large airway tissues, while complement activation was found in vascular tissues. The differentially expressed genes in the nasopharyngeal swabs correlated to tissue-specific gene expression in the early stages of infection but not in later infection. There was enrichment of macrophage, neutrophil, and T-cell pathways in SARS-CoV-2 samples compared to the non–COVID-19 acute lung injury samples. *(Article Selection: Jamie Sleigh, M.D. Image: Public domain, available at https://www.genome.gov/about-genomics/fact-sheets/Transcriptome-Fact-Sheet.)*

Take home message: The map of SARS-CoV-2 molecular pathophysiology is complex but dominated by transcriptional dysregulation of immune responses and loss of tissue identity.

 DOI: 10.1097/ALN.0000000000004246

Chronic paternal morphine exposure increases sensitivity to morphine-derived pain relief in male progeny. Sci Adv 2022; 8:eabk2425. PMID: 35171664.

Preclinical investigations report that prenatal morphine exposure may produce changes in the nervous system regarding morphine-induced antinociception in the offspring. However, previous approaches limit relevance of the results. This study evaluated consequences of prenatal morphine exposure (in the male parent) using novel approaches to assess nociception in rats validating a "rat pain scale" in both sexes using high-speed imaging of pain-like behaviors such as orbital tightening, paw shake, jumping, and paw guarding, combined with statistical approaches and machine learning. Analyses of behaviors obtained with von Frey filaments (commonly used to assess mechanical thresholds assumed to relate to nociception thresholds) did not produce pain-like behaviors. Offspring of morphine-exposed sires displayed morphine-induced antinociception that was detected with the rat pain scale but not with the latter. RNA sequencing in the periaqueductal gray indicated that morphine-sired male progeny had alterations in the expression of genes related to regulation of the G protein–signaling family of proteins with down-regulation in Rgs4, Rgs14, and Rgs16 and up-regulation in Rgs8. These proteins are known to regulate the opioid receptor, thus providing mechanisms in the alteration of morphine-induced antinociception in males prenatally exposed to morphine. *(Article Selection: Cyril Rivat, Ph.D. Image: J. P. Rathmell.)*

Take home message: This study demonstrates the need to develop more sensitive behavioral approach to accurately assess pain-like behaviors in animal studies. It also shows the effects of prenatal parental morphine exposure on offspring response to morphine-induced antinociception.

Ex vivo enzymatic treatment converts blood type A donor lungs into universal blood type lungs. Sci Transl Med 2022; 14:eabm7190. PMID: 35171649.

Successful lung transplantation depends on using donor organs that have protein or carbohydrate antigens on their cell surface that are immunologically compatible with the recipient. This results in some recipients (*e.g.*, ABO-O recipients) having to wait longer for compatible organs, increasing their risk of death due to lack of suitable donors. A strategy to create universally compatible ABO organs would improve fair access to donor organs. In the current study, human lungs were treated enzymatically to remove the A antigen during 1 to 3 h of *ex vivo* lung perfusion. After treatment, lungs were perfused with plasma samples from ABO-O individuals for 4 h to serve as a surrogate recipient circulation to mimic an *in vivo* reperfusion phase. Circulating anti-A antibodies were not depleted in the plasma, indicative of limited binding of antibodies to enzymatically ABO-A antigen-depleted cell surfaces. Inflammatory cytokines and complement C4d deposition were reduced in the enzymatically treated lungs compared to control lungs not devoid of ABO-A antigens. *(Article Selection: Charles Emala, M.D. Image: Adobe Stock.)*

Take home message: These preliminary findings suggest that enzymatic reduction of ABO-A antigens prevented hyperacute antibody-mediated injuries in simulated human lung transplantation; however, endogenous glycosyltransferases would be expected to regenerate cleaved A antigens such that longer-term compatibility requires further study.

Whole blood versus red cell concentrates for children with severe anaemia: A secondary analysis of the Transfusion and Treatment of African Children (TRACT) trial. Lancet Glob Health 2022; 10:e360–8. PMID: 35180419.

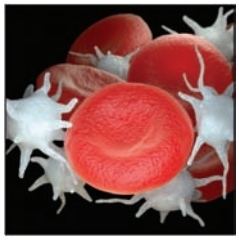
In sub-Saharan Africa, whole blood remains the first-line transfusion option for children with severe anemia although the use of components (*e.g.*, packed or settled red blood cells) is increasing. The Transfusion and Treatment of African Children (TRACT) trial was a factorial, randomized trial of children (2 months to 12 yr) with hemoglobin less than 6 g/dl conducted in Uganda and Malawi. Children received either 20 to 30 ml/kg whole blood or red cell concentrates *versus* no immediate transfusion (control group; unless hemoglobin was less than 4 g/dl). This secondary analysis examined the effects of whole blood *versus* red cell concentrates on outcomes in 3,188 children. Whole blood was the first component provided in 41% of the 3,992 transfusions. Hemoglobin recovery at 8 h was significantly lower in those who received component therapy than in those receiving whole blood (means ranging from –1.0 to –1.5 g/dl varying with packed *vs.* settled cells and dose [overall $P < 0.0001$]). Children receiving packed or settled cells had higher odds of a second transfusion (odds ratio, 2.32 [95% CI, 1.30 to 4.12] for packed cells and 2.97 [2.18 to 4.05] for settled cells; $P < 0.001$). There was no association between component type and mortality at 28 days or 180 days, or readmission to hospital. *(Article Selection: David Faraoni, M.D., Ph.D. Image: J. P. Rathmell.)*

Take home message: In anemic children in sub-Saharan Africa, transfusion with packed or settled red cells led to a slower rise in hemoglobin and greater odds of a second transfusion relative to whole blood transfusion.

A three-step support strategy for relatives of patients dying in the intensive care unit: A cluster randomised trial. The Lancet 2022; 399:656–64. PMID: 35065008.

One in five Americans are admitted to the intensive care unit (ICU) at or near the time of their death. This is a major stressor for caregivers, who experience high rates of depression, anxiety, and posttraumatic stress disorder during and after their relative's time in the ICU. This multi-center, cluster-randomized controlled trial conducted from 2017 to 2018 in 34 ICUs across France evaluated whether a physician-driven and nurse-aided three-step support strategy for caregivers who have decided to withdraw or withhold life support would improve caregiver outcomes. The intervention comprised three meetings of the attending physician and bedside nurse with the caregiver, at the time of the decision to withdraw, during the dying process, and after the patient's death. Staff were trained to emphasize attentive listening, empathy, and caregiver well-being. The primary outcome was the proportion of caregivers with prolonged grief (as measured with the Prolonged Grief-13 questionnaire, score 30 or above), measured 6 months after the patient's death. The study enrolled and randomized 875 caregivers, and 379 (78%) in the intervention group *versus* 309 (79%) completed 6-month follow-up. Caregivers in the intervention group were less likely to have prolonged grief (21% *vs.* 15%; $P = 0.035$) and had lower median Prolonged Grief-13 scores (19 *vs.* 21; mean difference, 2.5; 95% CI, 1.04 to 3.95). *(Article Selection: Meghan Prin, M.D., M.S. Image: J. P. Rathmell.)*

Take home message: A physician-led and nurse-supported three-step support strategy for caregivers who decide to withdraw life support for a dying relative resulted in less prolonged grief among caregivers.



Platelet-mimicking procoagulant nanoparticles augment hemostasis in animal models of bleeding. Sci Transl Med 2022; 14:eabb8975. PMID: 35080915.

The use of platelets for the treatment of bleeding disorders faces challenges, including bacterial contamination, donor availability, short shelf life, and high costs. Hence, the availability of a platelet-mimicking procoagulant for the management of bleeding would be beneficial. This study developed a hybrid liposomal nanoparticle system (platelet-mimicking procoagulant nanoparticles) in which the liposomal membrane contained distearoylated phosphatidylserine, the procoagulant anionic phospholipid found on the surface of activated platelets, in combination with other lipopeptide components, namely cholesterol-tethered polyethylene glycol, which is cleaved at the site of injury by plasmin, allowing site-specific activation of the clotting cascade. *In vitro* experiments with platelet-mimicking procoagulant nanoparticles immobilized on glass slides showed rescue of thrombin generation and clot formation in thrombocytopenic plasma. Addition of platelet-mimicking procoagulant nanoparticles to human plasma depleted of platelets amplified thrombin and fibrin generation and reduced clot lysis. *In vivo* experiments testing hemostatic effect of platelet-mimicking procoagulant nanoparticles in a tail transection mouse model where mice were rendered thrombocytopenic showed that 2 mg/kg platelet-mimicking procoagulant nanoparticles administered 2 h before tail transection reduced bleeding times from 1,071 to 404 s ($P < 0.001$), reaching bleeding times similar to those in conditions with platelet substitution (355 s). Platelet-mimicking procoagulant nanoparticles also enhanced hemostasis and survival in rodent traumatic hemorrhage models. *(Article Selection: Michael Zaugg, M.D., M.B.A. Image: J. P. Rathmell.)*

Take home message: Platelet-mimicking procoagulant nanoparticles foster hemostasis without off-target thrombotic risks in rodent models of bleeding.

Activity-dependent spinal cord neuromodulation rapidly restores trunk and leg motor functions after complete paralysis. Nat Med 2022; 28:260–71. PMID: 35132264.

Each year, nearly half a million people suffer from spinal cord injury, which can lead to severe disability and poor quality of life and is associated with a high mortality rate, even after surviving the initial injury. In this setting, epidural electrical stimulation—commonly termed *spinal cord stimulation*—recruits large-diameter afferent fibers entering the spinal cord *via* the dorsal roots, which in well-selected patients can improve motor function in incomplete injury to facilitate ambulation. This study demonstrates a redesigned paddle electrode to target dorsal roots involved in lower trunk and leg movements. A computational framework was created using structural and functional imaging to optimize lead placement with software enabling the rapid configuration of biomimetic stimulation programs that deliver concurrent stimulation waveforms that are switched on and off with precision. The device and software were tested in three individuals with complete sensorimotor paralysis and quiescent muscles during any attempt to walk. Within 1 day, these individuals regained the ability to control motor movements so that they could independently step on a treadmill, although extension motions were limited. Within 3 days, all could ambulate independently and cycle or swim. *(Article Selection: Steven Cohen, M.D. Image: Adobe Stock.)*

Take home message: Building on advances in neuromodulation in patients with incomplete spinal cord injury, spinal cord stimulation provides hope for clinically meaningful improvement in patients with complete injury and possibly a wide range of neurodegenerative conditions.

 DOI: 10.1097/ALN.0000000000004246

Twitch Monitor to Choose?

Quantitative Intraoperative Neuromuscular Monitoring

Quantitative train-of-four (TOF) monitoring is recommended after neuromuscular blocking drug (NMB) administration.[1] In this issue of ANESTHESIOLOGY, Weigel *et al.* describe a quality improvement project aimed at attaining documented quantitative TOF ratios ≥0.90 in all patients receiving NMB.[2]

300-bed urban teaching hospital with 28 anesthetizing locations

By combining principles of quality improvement and implementation science, evidence-based practices can be implemented in routine clinical care. Weigel *et al.* demonstrated >90% compliance with documented TOF ≥ 0.90, which persisted after project completion.

	PACU LOS	Hospital LOS	Pulmonary Complications	Sugammadex
	73 m [55,102]	3 days [2,5]	94 of 4,138 (2.3%)	1,377 of 9,034 (15%)
	68 m [49,95]	2 days [1,4]	23 of 1,817 (1.3%)	2,879 of 4,157 (69%)
	P<0.001	*P*<0.001	*P*<0.010	*P*<0.001

Department Professional Practice Initiative

Monitors
Selection based on:
- Clinician feedback
- Projected costs in all anesthetizing locations

Instructional videos
- How to place electrodes
- Underlying technology
- Device menus

Reminders
- Automated charting reminders
- Department & individual emails

Performance
- Ongoing Professional Practice Performance metric
- Tied to credentialing

LOS, length of stay; PACU, postanesthesia care unit.

*Percentage of cases with documentation of quantitative train-of-four ≥ 0.90.

Infographic created by Holly B. Ende, Vanderbilt University Medical Center; James P. Rathmell, Brigham and Women's Health Care/Harvard Medical School; and Jonathan P. Wanderer, Vanderbilt University Medical Center. Illustration by Annemarie Johnson, Vivo Visuals Studio. Address correspondence to Dr. Ende: holly.ende@vumc.org.

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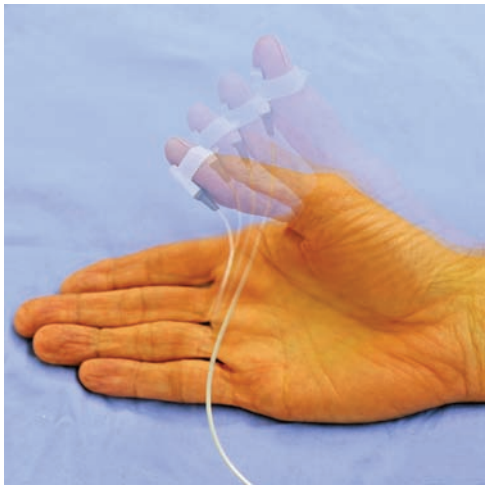
What Anesthesiology Has to Learn from Implementation Science and Quality Improvement

Meghan B. Lane-Fall, M.D., M.S.H.P., F.C.C.M.

In the 1960s, sociologist Everett Rogers puzzled over why farmers, when confronted with the opportunity to use superior seeds, decided to keep using the seeds they had always used. His observations formed the underpinnings of his diffusion of innovations theory, which explains how technologic innovations came to be adopted across populations.[1] Diffusion of innovations is one of the foundational theories of implementation science,[2] the discipline concerned with promoting the uptake of evidence-based interventions meant to improve health.[3] Perhaps, similar to Rogers, anesthesia professionals have puzzled over why evidence-based interventions in perioperative care—examples include the use of active warming during general anesthesia and the use of protocols and checklists to structure team processes—are not consistently used in practice.



"Unfortunately…the existence of evidence and creation of guidelines or recommendations are rarely sufficient to change practice."

In this issue of Anesthesiology, Weigel *et al.*[4] tackle the adoption of quantitative neuromuscular monitoring, an evidence-based practice shown to decrease the incidence of residual weakness after the use of nondepolarizing neuromuscular blockers.[5] As part of a robust professional practice change initiative, the authors used best practices in quality improvement to specify a change goal: documentation of train-of-four ratio greater than or equal to 0.90 for all patients. They documented compliance and other key outcomes over time, noting when key project milestones were reached to facilitate the attribution of change to specific actions by the quality improvement team. The authors used implementation strategies that seem well matched to the local contextual factors governing the use of quantitative neuromuscular blocker monitors. Specifically, they placed monitors in all operating rooms, selecting monitors based on feedback from clinicians and projected disposable costs; developed educational videos to instruct clinicians; instituted automated alerts using a customizable clinician support system; included neuromuscular blocker monitoring in their Ongoing Professional Practice Evaluation metrics; linked quantitative neuromuscular blocker monitoring to credentialing; and sent department and individual email messages about performance.[4] These strategies are consistent with those described in the Expert Recommendations for Implementing Change,[6] a compilation of implementation strategies that can be combined to facilitate individual and organizational behavior change. As a result of their efforts, the authors were able to achieve greater than 90% compliance with train-of-four documentation that persisted for more than 6 months. These changes were associated with decreases in postanesthesia care unit and hospital length of stay and a decrease in pulmonary complications.[4]

The use of nondepolarizing neuromuscular blocking drugs to facilitate intubation and surgery is second nature to anyone practicing anesthesia. We must use these drugs with care, however, because residual neuromuscular blockade after surgery has clearly demonstrated adverse outcomes for patients, including hypoxemia, a need for reintubation, and pneumonia.[7] Residual neuromuscular blockade is more likely when the depth of neuromuscular blockade is not monitored at all or when it is monitored qualitatively (*e.g.*, inspection and estimation of the train-of-four ratio) rather

Image: J. P. Rathmell.

This editorial accompanies the article on p. 901. This article has a related Infographic on p. A17. This article has an audio podcast.

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than quantitatively (as with acceleromyography or electromyography).[5,7] For this reason, in 2018, a panel of experts called for abandonment of qualitative and clinical tests of muscle strength in favor of quantitative monitoring.[8]

Unfortunately, as many of us know, the existence of evidence and creation of guidelines or recommendations are rarely sufficient to change practice. Weigel *et al.*[4] noted inertia with respect to the adoption and use of quantitative neuromuscular blocker monitoring in their institution and undertook a quality improvement initiative to change practice. In so doing, they designed a robust project that combines tenets of quality improvement and implementation science. This combination is not just interesting to the practicing anesthesia provider; it also offers insights into the deliberate selection and measurement of change strategies in anesthesia care.

Quality improvement and implementation science are similar in that they are focused on behavior change in organizations. They are different in their focus on the creation of local *versus* transferable knowledge (quality improvement is local); the explicit use of theories, models, and frameworks to guide study design, measurement, and reporting (implementation science relies heavily on theories, models, and frameworks); and the use of qualitative and mixed methods to understand context (implementation science commonly uses these approaches). Quality improvement is often funded locally, while implementation science has enjoyed increasing attention from research funding agencies that see the field as a way to realize the return on investment in basic and clinical research innovations. Despite the fields' differences, quality improvement– and implementation science–informed approaches to change management are not mutually exclusive; techniques from each can be combined to powerful effect, as demonstrated by Weigel *et al.*[4]

The astute reader will note that Anesthesiology does not often publish quality improvement reports. What do we have to learn from single-site experiences? From the implementation scientist's perspective, the answer is obvious. "Trusted Evidence: Discovery to Practice®" is in the journal's masthead. The evidence—here, the use of quantitative neuromuscular blocker monitoring—must reach practice to improve the care and outcomes of our patients. The report by Weigel *et al.*[4] provides insight into how that translation into practice might happen. It also demonstrates that change occurs over time and that sustained changes (*i.e.*, those lasting months to years) may require different strategies over time to reach a goal performance target. Of course, this practice-based evidence does not replace the more conventional, hypothesis-driven controlled trials that provide evidence of efficacy and effectiveness. Rather, these two types of evidence are complementary and reflect the complexity of modern anesthesia practice, which aims to continually improve patient care and outcomes.

Competing Interests

The author is not supported by, nor maintains any financial interest in, any commercial activity that may be associated with the topic of this article.

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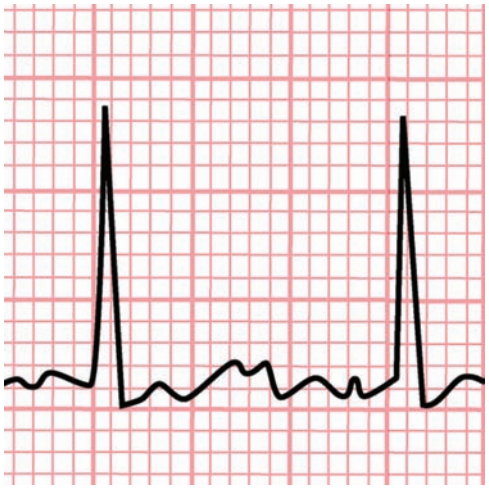
Inflammatory Responses to Surgery and Postoperative Atrial Fibrillation

Sergey Karamnov, M.D., Jochen D. Muehlschlegel, M.D., M.M.Sc., M.B.A.

Postoperative atrial fibrillation is one of the most common complications after thoracic noncardiac surgery. Despite contemporary prevention strategies, the rate of this complication can be as high as 40% in high-risk patients. Certain patient characteristics, including hypertension, obesity, smoking, male sex, and Caucasian ancestry, are known risk factors. Emerging evidence suggests that postoperative atrial fibrillation is not a transient benign phenomenon but rather an ominous postoperative complication associated with increased risk of thromboembolism, stroke, and mortality. Excessive inflammation associated with both tissue and extracellular free radical oxygen–mediated injury resulting from operative insult is thought to be an important contributing factor in postoperative atrial fibrillation pathophysiology. Anti-inflammatory strategies have been investigated for postoperative atrial fibrillation prophylaxis and demonstrated variable success.



"…postoperative atrial fibrillation is not a transient benign phenomenon but rather an ominous postoperative complication associated with increased risk of thromboembolism, stroke, and mortality."

In this issue of Anesthesiology, Amar *et al.*[1] studied the effect of *N*-acetylcysteine, a known antioxidant and free radical scavenger, as a prophylactic measure for postoperative atrial fibrillation after thoracic noncardiac surgery. The authors conducted a double-blind trial to compare amiodarone and the combination of amiodarone and *N*-acetylcysteine on the incidence of postoperative atrial fibrillation and postoperative stroke. They also examined postsurgery samples for serum markers indicative of systemic inflammation. No statistically significant differences were uncovered between the two patient groups in rates of postoperative atrial fibrillation or postoperative stroke or in the serum markers of ongoing inflammation. The study has been stopped in the interim analysis due to futility.

What could be a potential reason for no demonstrated benefits? The concept of atrial fibrillation being mediated by free radicals generated in the inflammatory milieu has not been clearly demonstrated in thoracic noncardiac surgery patients; therefore, *N*-acetylcysteine may not be an effective medication for these patients. An anti-inflammatory approach to postoperative atrial fibrillation is not a novel idea. A wide variety of medications with documented anti-inflammatory properties have been investigated for postoperative atrial fibrillation prophylaxis. Steroid and nonsteroid anti-inflammatory agents, ω-3 polyunsaturated fatty acids, inhibitors of the renin–angiotensin system, statins, and immunosuppressants demonstrated some success in human trials and animal models.[2] However, side effect–related considerations and a lack of strong reproducible benefits resulted in limited applications of anti-inflammatory agents in clinical practice. In fact, anti-inflammatory agents are not included in the most recent postoperative atrial fibrillation prophylaxis guidelines[3] and practice advisories[4] for cardiac and thoracic noncardiac patients.

Moreover, anti-inflammatory strategies *per se* may not be the ideal therapeutic direction to pursue. Inflammation entails way more than simply insult-triggered free radical

Image: J. P. Rathmell.

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formation and subsequent tissue damage. In fact, an acute inflammatory response is a host-protective, actively regulated, and, most importantly, normally self-regulating self-limiting process *in vivo*. Specifically, the onset of acute inflammation triggers production of signal molecules, such as specialized proresolving lipid mediators. Specialized proresolving lipid mediator networks serve as a link between mechanisms governing the onset and resolution of inflammation, resulting in insult mitigation, tissue healing, and restoration of homeostasis.[5] In contrast, uncontrolled inflammation and specialized proresolving lipid mediator production dysregulation may lead to excessive tissue damage, chronic inflammation, and deterred recovery as demonstrated in human and animal models.[6,7]

An anti-inflammatory approach for postoperative atrial fibrillation seems like a plausible strategy for postoperative atrial fibrillation prophylaxis. The documented association between postoperative atrial fibrillation and elevated serum concentrations of interleukin-6 and C-reactive protein, as well as higher postoperative leukocyte counts, suggests that inflammation may play an important role in the natural history of postoperative atrial fibrillation. This is further supported by the association between inflammatory conditions of the heart, such as myocarditis and pericarditis, and common atrial fibrillation. Exacerbated arrhythmogenicity of cardiac myocytes by elevated concentration of inflammatory markers due to the stresses of surgery is the likely mechanism of postoperative atrial fibrillation in susceptible patients.

More specifically, in thoracic noncardiac surgery, operative insult–related inflammation is triggered by a variety of different pathways related to one-lung ventilation, which is commonly exercised to facilitate the approach. First, ventilator-induced lung injury is a recognized complication after one-lung ventilation. The ventilated lung is subjected to oxidative stress and hyperemia-related capillary shear stress. Volutrauma due to increased, nonphysiologic tidal volumes and loss of the normal functional residual capacity further exacerbate lung injury.[8] Second, surgical insult itself results in additional mechanical tissue damage and subsequent local inflammation. Third, atelectasis in the nonventilated lung leads to hypoxic pulmonary vasoconstriction and local tissue ischemia. Lung reexpansion and oxygen reentry at the conclusion of one-lung ventilation result in reactive vasodilation and reperfusion. The resulting dissemination of inflammatory markers and oxygen free radicals generated by pulmonary parenchyma, one of the largest natural reservoirs of inflammatory cells, accelerates the systemic effects in target organs. Further work is needed to understand whether these mechanical insults during thoracic surgery are modifiable risk factors that can be attenuated with anti-inflammatory medications or whether they will require other therapeutic strategies, such as changes in ventilatory management.

So why did anti-inflammatory approaches demonstrate success after cardiac but not noncardiac thoracic surgery? Some key differences in cardiac and thoracic noncardiac surgeries may play a role. First, cardiopulmonary bypass utilized during cardiac surgery and the related inflammatory response have been implicated in the pathogenesis of postoperative atrial fibrillation in cardiac surgery patients. Systemic inflammation triggered by cardiopulmonary bypass and massive release of proinflammatory markers is thought to alter cardiac myocyte conduction. The degree of inflammatory response due to cardiopulmonary bypass likely supersedes the one due to one-lung ventilation during noncardiac surgery. In fact, the inflammation pathophysiology of nonventilated lungs applies to both lungs during cardiac surgery with cardiopulmonary bypass. The importance of an anti-inflammatory approach in cardiac surgical patients was recently addressed by Gaudino *et al.*[9] In this study, prophylactic intraoperative decompressive pericardiotomy to offset postoperative inflammation and swelling resulted in a lower incidence of postoperative atrial fibrillation. This study underscores the value of nonpharmacologic approaches to postoperative atrial fibrillation prevention and the value of multimodal therapy. Second, mechanical surgical insult to the myocardium triggers local inflammatory effects in the conduction system itself, contributing to increased incidence of postoperative atrial fibrillation after cardiac surgery. In contrast, postoperative atrial fibrillation after thoracic noncardiac surgery is thought to be triggered by a variety of different factors, such as dysregulation of the autonomic nervous system, age-exacerbated fibrosis, acute atrial stretch, and local pericarditis.[3] Much less is known about the role of local *versus* systemic tissue inflammation in the pathogenesis of postoperative atrial fibrillation after noncardiac thoracic surgery.[10] This may explain, at least in part, the scarce success of anti-inflammatory strategies for postoperative atrial fibrillation prevention in these patients. Similarly, despite the well documented effect of *N*-acetylcysteine on proinflammatory cytokines and oxygen free radicals on ischemia–reperfusion injury, as well as some success in cardiac surgery patients, the utility in postoperative atrial fibrillation prevention in thoracic noncardiac surgery patients may be limited.

In conclusion, the work of Amar *et al.*[1] demonstrates the feasibility and safety of *N*-acetylcysteine administration in this patient population. However, the study, which was stopped early at the interim analysis because of futility, discovered no additional benefit for the prevention of postoperative atrial fibrillation and its complications when compared to a conventional approach. Nevertheless, the emerging evidence of the long-term negative implications of postoperative atrial fibrillation that is further magnified by the unacceptably high rate of this complication calls for novel prophylactic strategies. The restoration of normal inflammatory pathways could be one of the potential therapeutic approaches for postoperative atrial fibrillation. Future

investigations can be directed at further elucidating the role of inflammation in the pathophysiology and pathogenesis of postoperative atrial fibrillation after thoracic noncardiac surgery to determine therapeutic targets.

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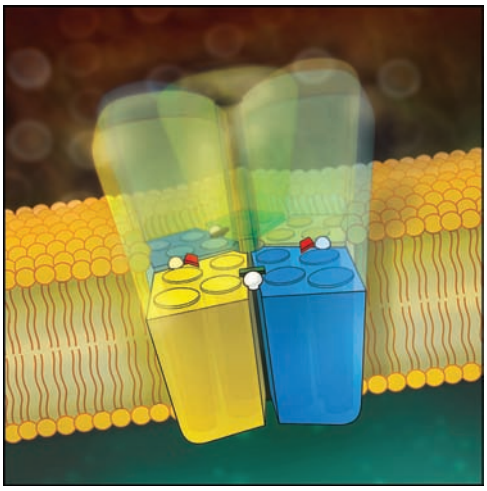
γ-Aminobutyric Acid Type A Receptor Subtypes and Circuit Connections in Midazolam-induced Amnesia, Sedation, and Hypnosis

Stuart A. Forman, M.D., Ph.D.

Midazolam is a benzodiazepine that at low doses is widely used in periprocedural care settings to assure amnesia and produce anxiolysis and sedation, while high doses are less frequently used to induce hypnosis (unconsciousness). Midazolam is also used to acutely suppress seizures. The molecular targets mediating these drug effects are γ-aminobutyric acid type A ($GABA_A$) receptors in the central nervous system, pentameric ligand-gated chloride channels that, when activated, hyperpolarize neurons and suppress their activation. This is the vastly oversimplified story we teach medical students, omitting many detailed scientific discoveries and unanswered questions that make the story of benzodiazepines fascinating, multifaceted, and open-ended. Important questions include the following: What types of $GABA_A$ receptors mediate the various neurobiologic effects of benzodiazepines? What are the relevant neural circuits mediating those effects? How are $GABA_A$ receptor subtypes distributed in those circuits and associated glial cells? Can pharmacologically targeting benzodiazepine sites selectively treat neurologic disorders? A new piece of that story is introduced in this issue of Anesthesiology in an article by Puig-Bosch *et al.*,[1] emerging from an international research collaboration. The elegant experiments described by Puig-Bosch *et al.* use tissue from transgenic animals containing mutations that eliminate midazolam sensitivity to explore the role of specific $GABA_A$ receptor subunits in neural circuits involved in amnesia, sedation, and hypnosis. The major findings, telegraphed in the title, implicate α1-containing receptors in midazolam effects on the neural circuits that were studied.



"…investigational compounds that selectively target a growing set of $GABA_A$ receptor allosteric modulator sites may evolve into future drugs…"

These experiments are based on knowledge gained from decades of research into benzodiazepine molecular sites of action and neuronal circuits, using an ever-growing set of research tools. The key experimental approaches scale from molecules to animals, spanning genetics, protein structural studies, electrophysiology, neuroanatomy, pharmacology, and behavior. The molecular pharmacology of benzodiazepines helps us understand the strategy used by Puig-Bosch *et al.* Nineteen $GABA_A$ receptor subunits are encoded in the human genome: α1 to α6, β1 to β3, γ1 to γ3, δ, ε, Φ, π and ρ1 to ρ3.[2] Among many thousands of possible pentameric subunit combinations, only a few dozen exist in neurons and glial cells in the central nervous system. Classical benzodiazepines like midazolam target typical postsynaptic receptors that contain two α subunits, two β subunits, and a γ subunit, arranged as depicted in figure 1. This configuration was first proposed based on functional electrophysiology studies of genetically engineered subunit assemblies that constrained their arrangement, and later confirmed with cryo-electron micrography.[3,4] Photolabeling and cryo-electron micrography locate benzodiazepine binding within the extracellular interface between the α and γ subunits, near a histidine at position 102 in α1.[5,6] These sites are structural homologs of the extracellular pockets where γ-aminobutyric acid binds between β and α subunits, paralleling evidence that benzodiazepines act as allosteric coagonists.[7] Electrophysiologic studies of $GABA_A$ receptors containing different subunits

Image: A. Johnson, Vivo Visuals Studio.

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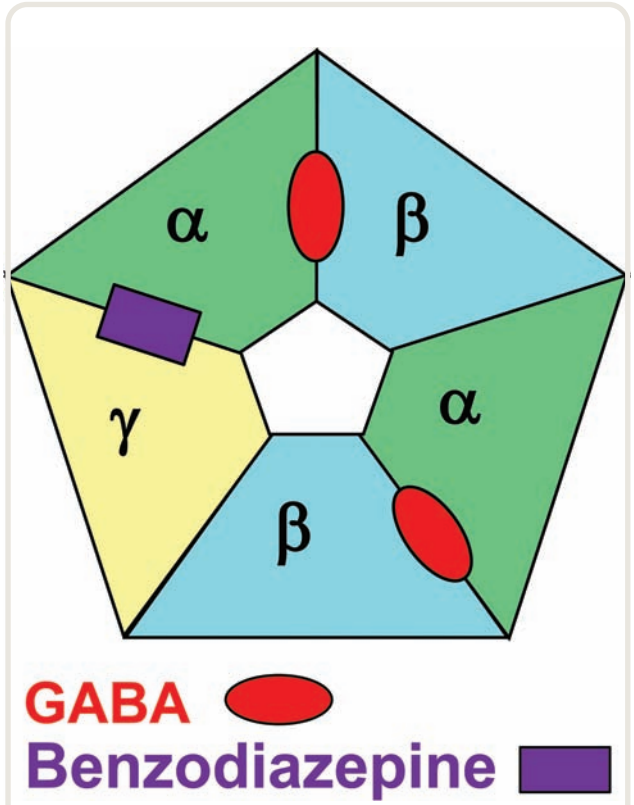

Fig. 1. A diagram depicting the arrangement of subunits in typical synaptic γ-aminobutyric acid (GABA) type A receptors and the extracellular intersubunit binding site locations for GABA and classical benzodiazepines.

revealed that classical benzodiazepines enhance activation when the α1, α2, α3, and α5 subunits, but not α4 or α6, are included. The benzodiazepine-sensitive α subunits all contain histidines at positions homologous to α1H102, while α4 and α6 contain arginine residues. Mutating these histidines in benzodiazepine-sensitive α subunits to arginines renders receptors benzodiazepine-insensitive.[8]

Members of the research collaborative created "knock-in" transgenic lines of mice with histidine-to-arginine mutations in each of the four benzodiazepine-sensitive α subunit types: α1-KI, α2-KI, α3-KI, and α5-KI. By cross-breeding these lines, they also created triple-mutant lines of mice with only one benzodiazepine-sensitive α subtype. Thus, in α1/2/3-KI, α1/3/5-KI, and α2/3/5-KI lines, respectively, benzodiazepines affect only α5, α2, or α1 receptors. From an interpretive standpoint, these knock-in lines are ideal for establishing whether a particular α subtype is both necessary and sufficient for a particular outcome. In that case, the single subtype knock-in should eliminate the benzodiazepine effect, while the complementary triple knock-in should retain benzodiazepine sensitivity.

These transgenic mice have previously been studied using various behavioral tests for distinct benzodiazepine effects, indicating that α1 receptors contribute to sedation, amnesia, and seizure protection, while α2 receptors mediate anxiolysis and muscle relaxation.[9] Other experiments support a significant role for α5 subunits in learning and memory as well as amnesia caused by benzodiazepines or etomidate.[10] Indeed, α5 is highly expressed in the hippocampus, which is part of the neural circuit responsible for memory formation. However, α5 receptors often contain δ subunits that are associated with increased γ-aminobutyric acid sensitivity, lack of benzodiazepine sensitivity, and extrasynaptic localization on neurons.[11] Thus, it remains uncertain which α subunit(s) are important for benzodiazepine-induced amnesia.

To bridge murine behavior with relevant neural circuits, Puig-Bosch *et al.* studied electrophysiologic effects of midazolam exposure in brain slices from wild-type and transgenic mice. The phenomenon known as long-term potentiation in hippocampal CA1 regions is an established correlate of memory. Long-term potentiation in hippocampal slices was observed as prolonged enhancement of field potentials in response to low-frequency stimulation after a brief high-frequency "training" activation of Schaffer collaterals in another part of the hippocampal circuit. For correlates of sedation (low-dose midazolam) and hypnosis (high-dose midazolam), decay rates of inhibitory postsynaptic currents were measured in voltage-clamped neurons in both hippocampal slices and cultured cortical slices. Here, the effect of midazolam in wild-type neurons is to reversibly prolong the decay of inhibitory postsynaptic currents, which are mediated by synaptic $GABA_A$ receptors. The results implicate α1 receptors, echoing previous behavioral studies, but also add a twist in the long-term potentiation outcomes, suggesting a novel model involving an interaction between other types of hippocampal $GABA_A$ receptors on different neurons in the circuit. Future studies should test this hypothesis. The postsynaptic current decay studies also need follow-up to further explore the roles of non-α1 receptors.

In vitro neural circuit experiments have both advantages and disadvantages relative to studies in live animals. A major advantage is that the experiments can be done with precise control of midazolam concentrations in fluid bathing the brain slices. In contrast, experiments in live mice cannot be done under conditions of steady-state benzodiazepine concentrations in the central nervous system. Pharmacokinetic factors (absorption, distribution, metabolism, and excretion) result in continuously varying concentrations after drug administration, and midazolam, a short-acting benzodiazepine, is particularly challenging. Limitations to these approaches include the high likelihood that other brain regions contribute to the behavioral effects of interest and that the relevant midazolam concentrations associated with various behavioral effects are uncertain.

The results of Puig-Bosch *et al.* represent scientific progress toward mapping $GABA_A$ receptors in neurons, glia, and neural circuits, connecting physiology and pharmacology to brain function and dysfunction.[11] Such work is of fundamental importance for understanding neurophysiology and relevant pharmacology. From a more practical perspective, $GABA_A$ receptors can be viewed as an important set of drug targets that play key roles not just in perioperative medicine but also in various neurologic disorders. Benzodiazepines, other benzodiazepine-site modulators, and investigational compounds that selectively target a growing set of $GABA_A$ receptor allosteric modulator sites may evolve into future drugs that provide subtle modulation at specific receptor subtypes. Examples include nonsedating nonamnestic anxiolytics and cognitive enhancers. Thus, this unfolding research story may influence the future of medicine.

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Optimizing Postoperative Analgesia in the Multiverse of Peripheral Nerve Catheters

Ellen M. Soffin, M.D., Ph.D.

Continuous peripheral catheters provide prolonged analgesia, reduce opioid consumption, and enhance patient satisfaction after painful surgeries.[1,2] Although these clinical benefits have been firmly established, the optimal strategy for catheter dosing regimens has not. Available options include continuous infusion, patient-controlled boluses, and pump-programmed intermittent boluses. To date, there is limited evidence to endorse one technique over another to improve clinical outcomes, and contemporary regimens sometimes include all three.[3,4] Adding to the complexity, the clinical advantages of modern pump technology and the regimens that can be reliably delivered are unclear.[4]

"[This is] the best evidence to date to support bolus-based regimens for popliteal–sciatic catheters after foot and ankle surgery."

The randomized clinical trial by Finneran *et al*.[5] in this issue of Anesthesiology elegantly addresses these important questions. The authors compared the effects of two different catheter dosing regimens on outcomes after ambulatory foot and ankle surgery. The study was powered for two primary endpoints: pain on postoperative day 1 and time until local anesthetic reservoir exhaustion. Additional outcomes included opioid consumption, sleep quality, and satisfaction with postoperative analgesia. Seventy patients received preincision popliteal–sciatic blocks, and a catheter was placed for postoperative use. Half of the patients were randomized to receive a continuous infusion of local anesthetic (6 ml/h) initiated before discharge, and the other half received automated boluses (8 ml every 2 h) initiated 5 h after discharge, using a start-delay timer. All patients had access to self-administered boluses on an as-needed basis. The automated bolus/delayed start regimen was hypothesized to produce at least noninferior analgesia and to conserve local anesthetic volume, thereby extending the duration of catheter use and analgesia. To their surprise, the authors found superior analgesia, lower median pain scores on postoperative day 1, 83% less opioid consumption, and significantly longer time to local anesthetic reservoir exhaustion (postoperative day 5) among patients randomized to the automated bolus regimen.

The results of the current study are compelling not just because they shed light on the nature of the optimal dosing regimen for home catheter use, but also because they provide a basis for future comparative research into the site- and procedure-specific applications for peripheral catheters. Previous studies comparing bolus with continuous regimens have yielded conflicting results.[3,4,6] Reasons for this are multifactorial and likely reflect anatomic differences at the target site, the techniques used to guide placement (ultrasound *vs*. nerve stimulation), the choice of local anesthetic and catheter type (stimulating *vs.* nonstimulating), and the outcomes assessed. The informing evidence base also suffers from substantial trial heterogeneity, small sample sizes, and lack of standardized interventions—all of which hamper our ability to conclude the superiority of any technique. This well-designed and adequately powered study is a clear methodologic advance over previous work and provides the best evidence to date to support bolus-based regimens for popliteal–sciatic catheters after foot and ankle surgery.

The results also provide a strategy to optimize and extend the duration of postoperative analgesia beyond that which can be conventionally achieved using a catheter-based system. A major limiter of the duration of catheter use (assuming proper function) is the volume of the local anesthetic

Image: J. P. Rathmell/A. Johnson, Vivo Visuals Studio.

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reservoir, and this may be exhausted before the satisfactory resolution of surgical pain. On the other hand, catheters are commonly placed with a single-shot block, and there is dubious value of using a catheter while the initial block is still providing analgesic benefits. Use of the delay start timer and low(er) volumes per dose neatly address these drawbacks of traditional dosing regimens.

Although the study answers several important questions, it raises several more. The clinical significance of the outcomes needs to be confirmed, placed in the context of the recovery trajectory after foot and ankle surgery, and compared to other techniques in this surgical population. Notable by its absence is a comparator arm in which patients received single-shot popliteal–sciatic block (with adjuvants or long-acting local anesthetic) and comprehensive multimodal analgesia. The assumption—albeit supported by a wealth of published data[1]—is that any catheter is at least non inferior to a non–catheter-based analgesic regimen. What remains to be addressed is whether a 5-day duration of regional analgesia is necessary or sufficient for optimal recovery or represents a substantial advantage over other techniques in this surgical population.

Indeed, since all patients had access to self-administered boluses of local anesthetic to "top up" their catheter as desired, it is unclear why there were *any* differences in pain scores between the groups in the early postoperative period (or at least up until reservoir exhaustion in the continuous infusion group). The authors' interpretation is that patients were "saving" their local anesthetic to maximize the duration of the catheter use. An alternative explanation is that higher pain scores did not represent meaningful differences in recovery status or pain states, and patients did not need to lower their pain scores further by administering a local anesthetic bolus. Provocatively, this interpretation may be supported by the data regarding other secondary outcomes: total opioid consumption was significantly different between the groups, but impressively low in both. Likewise, patient-reported satisfaction scores were statistically higher among patients randomized to automated boluses at postoperative days 1 and 4, but the absolute scores for satisfaction were very high in both groups at all times.

Nonetheless, the prolonged duration of analgesia achieved with conservation of local anesthetic volume is impressive and suggests that pump technology that can deliver automated bolus dosing with delayed start infusions could become the new standard. The authors conclude that their results represent a paradigm shift in catheter-based care. This is likely to sound humbling to those who work in settings without catheter services and bold to those who do. Moreover, what should the clinician practicing in a setting without a catheter program take away from the study? The question is somewhat rhetorical but highlights the persistent gap between centers that have and those that do not have catheter programs. In this regard, it is as if we are living in a multiverse of peripheral catheters, where a few centers advance the care and research informing optimal use, even fewer do so *via* ambulatory-based programs, and most have no program at all. Other than an aspirational message, the results presented do not advance this issue or help to expand the benefits of catheters to more patients.

The research by Finneran *et al.*[5] is a welcome addition to the body of work directed toward improving recovery and outcomes after painful surgeries. The work represents a significant step forward in understanding the anatomic, site-specific properties of catheters and the infusion systems required for targeted analgesia. What remains to be achieved—more than 70 yr after the first description of a continuous peripheral nerve catheter[7]—is an understanding of the barriers to the broader adoption of catheter programs and their suitability for nonspecialist practices.

Competing Interests

The author is not supported by, nor maintains any financial interest in, any commercial activity that may be associated with the topic of this article.

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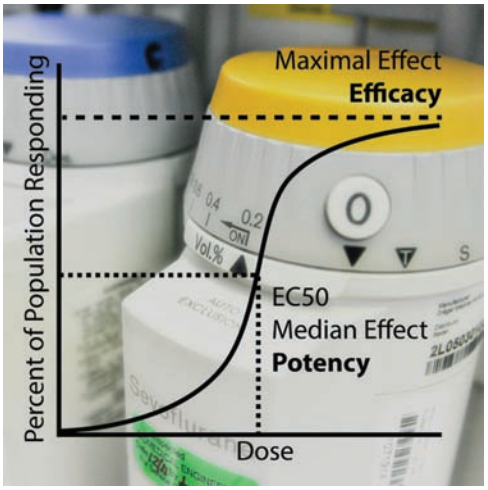
Anesthetic MAC: Origin, Utility, and Nomenclature Revisited

Evan D. Kharasch, M.D., Ph.D.

Anesthesiology is a specialty grounded in basic and clinical pharmacology. These domains have concepts and vocabularies that are useful to scientists and clinicians, in their understanding and application of pharmacology. Potency (drug dose or concentration producing a given effect) and efficacy (maximum drug effect) are two concepts that ground pharmacology. One of the venerated concepts and terms in anesthesiology is "MAC." MAC is a *nom de plume* for potency and EC50 (*vide infra*). Among the entire pharmacologic armamentarium, inhaled anesthetics are the only drugs for which potency has a special name, and inhaled anesthetics are the only ones in anesthesiology—indeed in all of medicine—that are dosed as a fraction of their EC50. As we celebrate the 60th anniversary of MAC, it is useful to examine what it means and what it stands for.



"It is time to call a MAC (minimum alveolar concentration) a MAC (median alveolar concentration)."

Last month in Anesthesiology, Dr. Larry Saidman, former Editor-in-Chief of Anesthesiology, recounted in a Classic Papers Revisited article[1] his role in the first study to determine MAC in humans.[2] He and Dr. Edmond "Ted" Eger II anesthetized 68 surgical patients with halothane to produce a light plane of surgical anesthesia, recorded the end-tidal halothane concentration, and observed whether the patients showed a muscular response to surgical incision. The halothane concentration at which half the patients responded with movement and half did not (the "transition point") was termed the MAC.[1,2] As described by Dr. Saidman, "The discovery of MAC in humans was revolutionary for clinical and research purposes in that it allowed the pharmacologic effects of inhaled anesthetics to be compared against each other at a similar anesthetic depth." Classic pharmacology.

This month in Anesthesiology, Drs. Jan F. A. Hendrickx and Andre M. De Wolf explicate the foundations of MAC, the underlying physiology and pharmacology, the clinical application, and the terminology in a Clinical Focus Review.[3] They explore definitions and determination of MAC, types of MAC (MAC_{awake}, $MAC_{unconsciousness}$, $MAC_{immobility}$, MAC_{BAR}), factors affecting MAC, fraction of a MAC delivered to a patient (fMAC), relationship between fMAC and clinical effect, and drug interactions or other factors affecting fMAC. They also present pragmatic approaches to using MAC and fMAC in clinical care. This comprehensive and lucidly written review provides both text and illustrations to visualize and reinforce the concepts. It is recommended to trainees as well as experienced clinicians.

Astute readers will detect that while this essay has described MAC, it has not yet defined it. For this, we need a bit of pharmacologic grounding (fig. 1). There are two types of dose (or concentration)–response curves. Graded dose-response curves describe drug response along a continuous scale (0 to 100% of maximal response—termed efficacy) in a single unit (*e.g.*, cell, organ, animal, or human). Quantal or population dose-response curves describe the fraction of a population of units responding with an all-or-none response (*e.g.*, awake or not). The amount of drug needed to produce 50% of a maximum effect in one unit is the ED_{50} or EC_{50}. The amount of drug needed to produce a quantal response in 50% of a population is the ED50 or EC50 (also known as the median dose or concentration). Both EC_{50} and EC50 describe drug potency (amount of drug needed for half-maximum effect), but they are clearly determined in very different ways. Now, back to MAC.

Even before Drs. Saidman and Eger determined the MAC of halothane in patients, Drs. Merkel and Eger first introduced the concept of MAC.[4] They determined the MAC of halothane in dogs. In each of six dogs, they determined

Image: J. P. Rathmell.

This editorial accompanies the article on p. 985.

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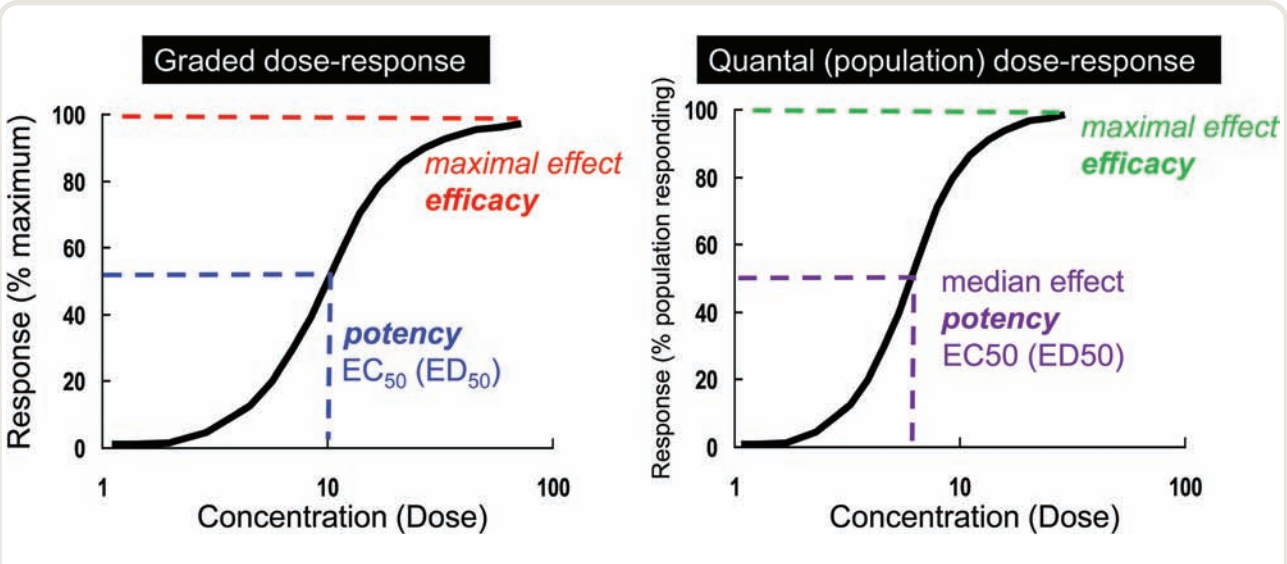

Fig. 1. Types of concentration (or dose)–response curves. (*Left*) Graded curve, showing percentage of maximum response (efficacy) in a single unit (*e.g.*, cell, organ, animal, or human). The dose (or concentration) of drug producing a half-maximum effect is the ED_{50} or EC_{50}. (*Right*) Quantal curve, showing the percentage of a study population showing a response to an all-or-nothing outcome. The dose (or concentration) of drug needed to produce a quantal response in 50% of the population is the ED50 or EC50 (also known as the median dose or concentration).

the *minimum* end-expired halothane concentration required to keep the dog from responding with gross movement to a painful stimulus (tail clamp or electrical stimulus). This was termed the "minimal anesthetic concentration" (1 MAC). Multiple, higher concentrations of halothane were also evaluated. The average MAC was determined from individual experiments in the six dogs. This was a classical, single-unit dose-response experiment, and truly determined the effective *minimum* anesthetic concentration. Later, in an analogous experiment, Drs. Saidman and Eger anesthetized four surgical patients, each one at multiple halothane concentrations, and determined the end-tidal halothane concentration that just eliminated movement to an electrical stimulus.[2] This was termed the "minimum alveolar concentration" (1 MAC). The average MAC was determined from the four patients. This too was a classical, single-unit dose-response experiment and truly determined the *minimum* anesthetic concentration. Drs. Saidman and Eger also did an experiment in which they anesthetized 68 surgical patients with halothane, each at a *single* concentration, and recorded the absence or presence of a muscular response to skin incision. The halothane concentration at which half the patient population moved and half did not was termed the "minimum alveolar concentration" (1 MAC). However, this was a classical population *quantal* dose-response experiment that did not determine the *minimum* effective concentration, but rather the *median* effective concentration. A totally different construct, yet the same term (and abbreviation) were used to refer to both individual (minimum) and population (median) values—only one of which is correct.

Drs. Hendrickx and De Wolf identify that the term "MAC" is thus plagued with semantic issues.[3] Should the "M" in MAC refer to "minimal" or "median"? In addition, while alveolar and end-tidal terms have been used interchangeably, they also point out that alveolar and end-tidal anesthetic concentrations (what we actually measure) may not be the same. They propose that MAC should be redefined ("backronymed") as the "median alveolar concentration." Excellent suggestion. Or even more precisely, perhaps should it be redefined as the "median end-tidal anesthetic concentration": METAC? They also ask whether the acronym "MAC" should be abandoned altogether and replaced with the more universal EC50, because EC50 is conceptually and semantically more correct and aligns volatile anesthetic terminology with that of intravenous anesthetics and all other drugs. They suggest that MAC should not be abandoned, largely for practical reasons. This is because MAC describes anesthetic potency in a unifying manner across different anesthetics, allows the same anesthetic machine alarm limit (fraction of a MAC) to be applied to all volatile anesthetics, and is already hard-wired into anesthesia machine displays. Good point. Moreover, the use of MAC may contribute to patient safety.

MAC has withstood the test of time. It remains conceptually accurate, clinically useful, and helpful to the practicing clinician. And yet, in an era of precision medicine, we should use more precise terminology. It is time to call a MAC (minimum alveolar concentration) a MAC (median alveolar concentration).

Competing Interests

Dr. Kharasch is Editor-in-Chief of Anesthesiology.

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ANESTHESIOLOGY

Gadgeteering for Pain Relief: The 2021 John W. Severinghaus Lecture on Translational Science

James C. Eisenach, M.D.

ANESTHESIOLOGY 2022; 136:888–900

ABSTRACT

In this first memorial lecture after John Severinghaus's death in 2021, the author traces his journey as a physician–scientist, using the framework of the hero's journey as described by the author Joseph Campbell 40 to 50 yr ago, and parallels that journey to his own. The author discusses how each were gadgeteers: Severinghaus in a creative engineering way, while the author's approach was asking simple questions translating basic research in pain from animals to humans. The classic hero's journey of departure to achieve a goal, then trials, transformation, and finally, returning with benefits to the individual and others is translated to the common physician–scientist career with motivations progressing from "I will show" to "I wonder if" to "I wonder why." Critical to this journey is self-questioning, openness to new ideas, and realizing that progress occurs through failure as much as success.

(ANESTHESIOLOGY 2022; 136:888–900)

I thank the American Society of Anesthesiologists (ASA; Schaumburg, Illinois) for creating this opportunity each year to honor John Severinghaus and to do so this year a few months after his death[1] as a memorial to him. The University of California, San Francisco, has established an endowment to support Severinghaus professors in the Department of Anesthesia and Perioperative Care, and the ASA agreed for me to present this link (http://tiny.ucsf.edu/SeveringhausEndowment) if you would like to contribute. I was lucky enough to hear Dr. Severinghaus in 2008 give the first lecture bearing his name, in which he discussed his accomplishments, using the whimsical term of "gadgeteering." I thought it appropriate to organize my lecture in a similar fashion, as I, too, have been a tinker or gadgeteer, albeit with much less impact than John. This lecture and article are intended also to impart some thoughts for those who are now starting out as physician–scientists. It is organized as a three-act story with a brief prelude and a briefer postlude. It's a story about John Severinghaus, about me, and about you, too, whether or not you do research.

Prelude

This structure of the lecture was based on lessons from *Joseph Campbell and The Power of Myth*, a 1988 Public Broadcasting Service documentary series in which Bill Moyers interviewed Joseph Campbell, an author and scholar of myths across time and cultures.[2] Filmed at George Lucas's Skywalker Ranch, where Campbell had helped Lucas in creating the first *Star Wars* film, it's a fascinating conversation and well worth seeing. Campbell kept returning to what he termed the hero's journey, a series of events that are remarkably similar in the life stories of the Buddha or Shirley Chisholm or Ulysses or Jean Valjean or Jesus, and in books and films for children and adults today.

One of the most common hero's journeys can be described largely in three phases (fig. 1A). The first starts with leaving the comfort of the familiar to achieve a goal. Next, the hero overcomes, aided by strangers, mentors, and the experience of many trials, and is transformed through these events—in some cases without even achieving the goal. Finally, the hero returns, bringing new life to society through the hero's own vitality and experience of life's mystery. You and I are not mythical heroes, but we can follow similar steps in aspects of our lives, including our working careers.

As an example, consider the plenary lectures at the 2008 ASA Annual Meeting, which appeared as articles in the April 2009 issue of ANESTHESIOLOGY and were highlighted on the cover. Ronald Miller, M.D., went to Vietnam, returning with leadership and new knowledge of resuscitation and pharmacology to advance the specialty. Steven Shafer, M.D., went out to mathematical modeling, returning with concepts shaping drug development across medicine. And John Severinghaus, M.D., went on to become a physicist, returning a physician with devices that transformed medical care. Herein, I describe my own journey in parallel with that of Severinghaus and invite you, the reader, to consider your own personal journeys as we progress together.

We are a remarkably young specialty. Dr. Ralph Waters was founding our specialty when John Severinghaus was a boy. This is how John remembered Dr. Waters: "He [Dr. Waters] lived about two blocks from us in Madison. I played with his kids and his dad. His office was next to my dad's office at the university. Between the two, there was

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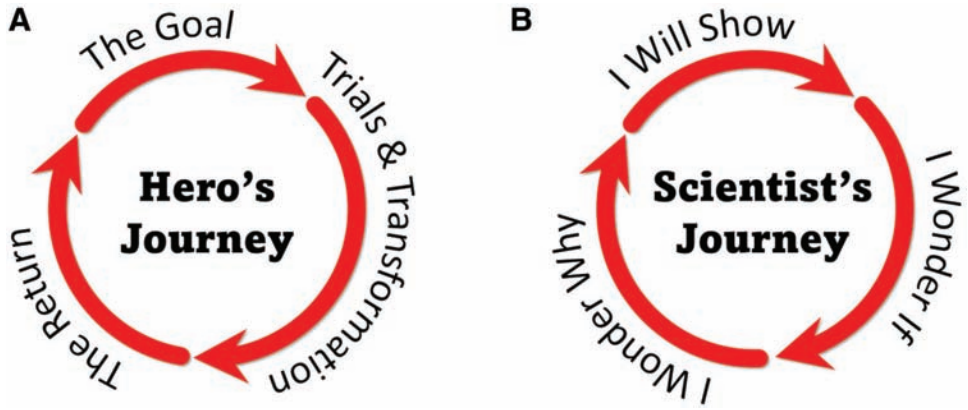


Fig. 1. Stages in (*A*) a hero's journey, beginning with The Goal and (*B*) a scientist's journey, beginning with I Will Show. The circle implies that these stages may be repeated.

a drinking fountain, a bubbler, as we called it in Madison. And one of my very early memories was of Ralph Waters taking a drink with his pipe still in his mouth from the bubbler between the two offices."[3] Physicians from around the globe came to Madison, Wisconsin, to study under Ralph Waters and returned to establish, as chairs, new departments of anesthesiology, birthing the specialty.

Figure 2 depicts some of the characters at the start of the story. In his 30s, John Severinghaus was recruited into anesthesiology by Robert Dripps, M.D., at the University of Pennsylvania (Philadelphia, Pennsylvania) and was subsequently recruited to the University of California, San Francisco, on the condition that an independent Department of Anesthesiology be created, headed by Stuart Cullen, M.D. As a resident in my 30s, I planned on entering private practice until the morning I heard Tony Yaksh, Ph.D., present an early morning grand rounds lecture at the Mayo Clinic (Rochester, Minnesota). He's still my mentor. My first chair, Francis M. James III, M.D., remains a mentor, sponsor, and role model to me to this day. This article shows images of many old white men mentioned in this talk, some of them young who became old. This reflects our specialty's history and my life experience in a position of privilege in our caste system. I should have done better.

Act I: I Will Show

Act I is all about achieving a goal. It begins on a clear day, and the goal, obviously requiring hard work to reach, is visible and the paths to it clear. For many early-stage scientists and certainly for me, the goal translates to "I will show" (fig. 1B). The focus is to prove a concept or create something to address a problem. Gadgeteering is most evident in Dr. Severinghaus's career during this phase. In the early 1950s, he invented an esophageal probe for physiologic monitoring, which he considered to be an unfortunate commercial flop.

The rationale for other gadgets during this period came from urgent needs presented by the polio epidemic. As he states in the Wood Library-Museum of Anesthesiology interview with Thomas Hornbein,[3] "I had built an electro-phrenic respirator the last year of medical school after watching Stan Sarnoff demonstrate how he could stimulate his wife's phrenic nerve just by touching the surface of her neck with this probe. I thought that would be a great way to do artificial respiration. This was really before we had much in the way of ventilators: the iron lung. It was a polio epidemic and that looked like a great bet for doing artificial ventilation. […] The three electrodes and the three techniques in blood gases, all in a sense came from this common need to measure blood, oxygen, CO_2 and mostly in the face of the polio epidemic." After decades of maiming and killing children and young adults, the polio epidemic ended with near universal vaccination, a true miracle of modern medicine. Rapid blood gas analysis went on to fundamentally advance perioperative and critical care medicine.

While researching this lecture, I've come across many stories about Severinghaus the gadgeteer. My favorite, contributed by the Swedish anesthesiologist Torsten Gordh, Jr., was Severinghaus's construction of a suspended pipe and an electric toy train to ferry arterial samples from a third floor operating theater to the laboratory, also on the third floor, in an adjacent building in Leeds, United Kingdom, for Professor John Nunns.

In my own Act I, I planned to show that α2-adrenergic agonists could treat labor pain safer than existing methods. Lumbar epidural analgesia using local anesthetics was the primary technique during my medical training, but case reports of untreatable cardiac arrest[4] and paralysis[5] appeared, leading to new Food and Drug Administration (Silver Spring, Maryland) regulations and drug withdrawals. Also, case reports of respiratory arrest and death from neuraxial

Fig. 2. John Severinghaus (*top left*) and James Eisenach (*bottom left*) in their 30s and the individuals who recruited them to scientific careers in anesthesiology and the original chairman who supported them. Reprinted with permission from the American Society of Anesthesiologists and the Wood Library-Museum of Anesthesiology (Schaumburg, Illinois).

opioids began to appear.[6] I submitted a grant proposal to the National Institutes of Health (Bethesda, Maryland) as a resident, arguing that α2-adrenergic agonists might be safer drugs to administer for obstetric anesthesia. That grant (No. R23GM35523 [Epidural Clonidine Analgesia in Obstetrics: Sheep Studies]) was funded during my obstetric anesthesia fellowship, and continued for the next 27 yr.

I was initiated into basic science in perinatology in the laboratory of James Rose, Ph.D., Professor of Obstetrics at Bowman Gray School of Medicine (now Wake Forest School of Medicine [Winston-Salem, North Carolina]). The commonly used animal model for obstetric physiology research at the time was pregnant sheep: probes and catheters were inserted to assess uterine blood flow and maternal and fetal hemodynamics and arterial blood gases. We used a Radiometer (Copenhagen, Denmark) arterial blood gas machine that was only a couple of generations beyond the one John Severinghaus first created, one of which currently resides in the Smithsonian Institution (Washington, D.C.). We described drug transfer from the ewe to the fetus and disposition of clonidine in plasma and cerebrospinal fluid (CSF) after epidural, spinal, and IV administration. This led to interactions with Donald Stanski, M.D., Chair of Anesthesiology at Stanford University (Stanford, California), and Steven Shafer, a friend and colleague ever since. Clonidine produced minimal or no hypotension in pregnant and nonpregnant sheep, so I showed what I had hoped to show.

At this time, I also received a grant from the orphan products division of the Food and Drug Administration in which I planned to show efficacy of epidural clonidine in humans. I would have failed if not for the intervention of two strangers, a classic event in the hero's journey. After obtaining investigational new drug approval, we began a randomized, controlled trial of one dose of clonidine for analgesia after surgery. My first study patient had new-onset atrial fibrillation, which resolved spontaneously in the intensive care unit. I called the orthopedic surgeon,

Gary Poehling, M.D., to say we were stopping the study. He felt I had no reason to believe this was study-related and encouraged me to continue the study, which I did after Food and Drug Administration review. After the first three subjects in the study had no analgesia, I was again ready to stop. While stewing over this, I traveled to Hamburg, Germany, to present some of our sheep research, and Tony Yaksh invited me to discuss science over a beer at a nearby pub. We were joined by a world-renowned pharmacologist from Memorial Sloan Kettering Cancer Center (New York, New York), Charles Inturrisi, Ph.D. He asked whether any of the patients had side effects from the dose of clonidine I had chosen, and I said no. This brought a hearty laugh and a 30-min explanation of the concept of dose-ranging studies. I returned home, redesigned the study, and showed dose-dependent analgesia from clonidine.[7]

Unfortunately, clonidine turned out to be a complete flop for obstetric anesthesia. Spinal clonidine does produce pain relief for 90 to 120 min in women during labor, as does epidural clonidine after cesarean delivery, but analgesia is accompanied by unacceptable hypotension and sedation.[8,9] Clonidine was an equal flop in nonpregnant patients after surgery.[10] In contrast, in patients with chronic regional pain syndrome, infusion of lumbar or cervical epidural clonidine for lower or upper extremity disease reduced pain compared to placebo from severe to moderate levels and was well tolerated.[11] In a randomized, controlled clinical trial in patients with intractable cancer pain, epidural infusion of clonidine produced sustained analgesia that was sustained for weeks,[12] leading to Food and Drug Administration approval. However, the Food and Drug Administration appropriately added a black box warning stating that "[E]pidural clonidine is not recommended for obstetrical, postpartum, or perioperative pain management." I clearly did not show what I wanted, but this work and that from others did expand the use of α2-adrenergic agonists from nasal decongestants and antihypertensive pills to a new, nonopioid treatment for cancer pain, a useful sedative, and an adjunct to regional anesthesia.

Severinghaus had a mind that was open to new ideas: "I began to look for easier ways to measure CO_2. I went to the physiologic society meeting in Madison in 1954 and heard Richard Stowe describe a carbon dioxide electrode and it clicked just like that, and I said, that's what I need."[3] During Act I, I too heard just what I needed at meetings, including two international meetings I organized about α2-agonists. Lawrence Saidman, M.D., invited me to join the Associate Editorial Board of Anesthesiology, and others must have recommended me to serve on National Institutes of Health grant review committees and an Food and Drug Administration Advisory Board and to give plenary lectures at international venues. I suspect Dr. James opened doors at the American Society of Anesthesiologists, the Association of University Anesthesiologists (San Francisco, California), and the American Board of Anesthesiology (Raleigh, North Carolina) and probably others. Our research group was small, with mostly European physicians, including Astrid Chiari, M.D., who gave birth to her first child when we were at the World Congress in Vienna, Austria. She later became Chair of the Department of Anesthesiology at the University of Vienna. Barry Seltzer, M.D., a musician and internist, and I established a book group of two every Tuesday night that has lasted to this day as we help each other navigate different stages of career and life. Like John Severinghaus, I took a sabbatical during this time, with 6 months in Iowa City, Iowa, where I became known as Sweet Baker James as I woke up early to bake sweets for the graduate students and fellows in the laboratory, then 6 months in Paris, France—a truly fantastic time for both career and family.

Act I involved what appeared to be mostly serendipity, like the two strangers who helped me in that first clonidine study. Just 2 weeks ago, I serendipitously came upon that orthopedic surgeon as I was bicycling home. I hadn't seen him in more than 20 yr, and took the opportunity to tell him the role he played in my life. Act I also involved many "invisible hands," a term Bill Moyers mentioned, to which Campbell replied, "When you begin to deal with the people who are in the field of your bliss they open doors to you." I will return to the concept of bliss later. Taking time such as a sabbatical is critical in Act I to truly experience and reflect on this bliss that is central to your journey.

Act II: I Wonder If

Act II is when the path becomes murky, dangers occur, and one can get lost or discouraged. It's moving away from being confined inside a rigid box filled with unconscious biases in "I will show…" toward a freer space of, as Steve Shafer likes to say, "letting the data speak." In both of our journeys (Severinghaus's and my own) during this phase, we examined spinal fluid; he was seeking an understanding for adaptation to high altitude, and I was manipulating spinal cord processing on pain transmission.

To do this, we gadgeteer-ed on ourselves as well as others. John Severinghaus's early work involved spinal fluid sampling, as well as respiratory measurements and arterial and jugular venous sampling, at the University of California Barcroft High Altitude Laboratory (Bishop, California). He described his postdural puncture headaches and his approach to prophylactic treatment: "I think I've had a headache with every needle tap except two. One of them was done at night with me lying face down. And I never turned over so that the needle hole was the highest thing in me from that time on all night. And I didn't get a headache after that one, but I tried that a few more times. Didn't work! And finally, when I was doing another one of these, must have been in the mid-1970s, I had 1 cc of blood taken from a vein and injected into the needle into the CSF as the needle was being pulled out to leave some fibrin inside. Didn't get any headache after that one!"[3] In my own case, I opted for the more conventional epidural injection of blood to patch the hole and relieve the headache.

Spinal Drug Distribution

As a byproduct of the work during this period, we studied spinal drug distribution, in part to better understand the pharmacology of novel spinal agents and in part to address a perplexing clinical observation with spinal anesthesia. With similar patients and injections, distribution of spinal anesthesia is rapid and extensive in most, but slow and limited in some. This has resulted in manipulations of injection method, volume and baricity, patient positioning, and maneuvers like coughing or straining to alter distribution. As a resident, I noticed that each attending had a special recipe they swore by, arguing, like confident chefs, that all others were wrong. This didn't seem very satisfactory.

We solicited the help of Steve Shafer and included pharmacokinetic/dynamic modeling in several studies of drugs injected neuraxially. For opioids, we inserted a needle in a low lumbar interspace and another more cephalad, injected fentanyl through the caudad needle, and sampled through the cephalad needle as drug spread from the injection site during the next 2 h with both needles in place (fig. 3). The average CSF fentanyl concentration from the cephalad needle (black line) peaked by 15 min and then slowly declined (fig. 3; black line). One individual (fig. 3; red line) had near-maximal concentrations at 1 min after injection. This very rapid movement is a good thing with local anesthetics, but could underlie cases of acute respiratory depression 10 to 20 min after spinal injection of lipophilic opioids during labor.[13] Another individual (fig. 3; green line) had no fentanyl in CSF from the cephalad needle until 4 min after injection, and the fentanyl concentration in CSF at 10 min was 40-fold lower than the one shown in red. This could predict the maddeningly slow distribution of spinal anesthesia with local anesthetics in some cases, making all of us anxious—especially the patient.

Steve Shafer successfully modeled these curves with a mixing kinetic constant.[14] Every individual had a different constant and we could not predict it from subject size, sex, age, or lumbosacral CSF volume measured by magnetic resonance imaging. We wondered if something else might predict it. CSF circulates in the cranium from the third ventricle around the cortical surface, where it is absorbed by arachnoid villi. Textbooks describe a similar lazy river–like flow of CSF in the spinal space, but there is little evidence for such bulk flow. Rather, studies dating back more than 50 yr suggest that CSF pressure fluctuates with each cardiac cycle[15] and that CSF jostles up and down, like water under a toilet plunger, as it is displaced from the cranium when blood volume increases then decreases. We thought that someone with a larger cardiac stroke volume might have faster and more extensive drug mixing in CSF than another person with a smaller stroke volume, and showed that the duration of spinal fentanyl analgesia during labor increased as stroke volume, assessed by pulse pressure around the time of injection, also increased.[16] This hypothesis deserves further testing.

We also explored drug distribution and action of novel drugs in spinal analgesia. For clonidine, we determined the transfer rate from the epidural space to CSF, generated computer-controlled epidural infusions to target constant CSF concentrations, and calculated CSF concentrations associated with analgesia.[10] For neostigmine, we injected a drug in a lumbar interspace, leaving the needle in place for 2 h for CSF sampling; Steve Shafer successfully modeled the spinal cord analgesic effects in the foot and in the hand from CSF levels at the injection site, using a concept he called observation from a distance.[17] The only problem was that every individual had a very different CSF concentration curve, making it useless for clinical prediction.

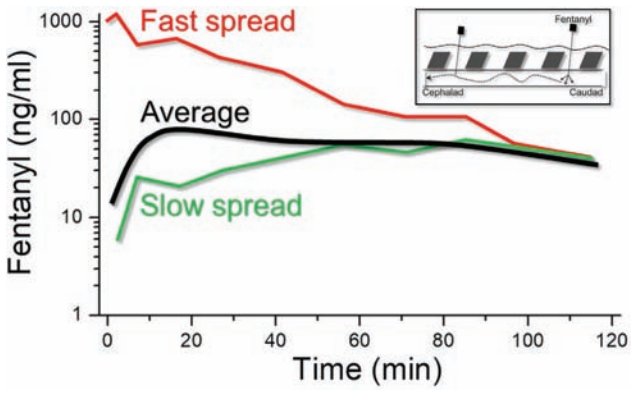


Fig. 3. Drug disposition in cerebrospinal fluid after intrathecal injection. (*Inset*) Study design showing insertion of two spinal needles: injection through one and sampling over time *via* the other. Cerebrospinal fluid concentrations through time: on average (*black*) and in individuals with fast (*red*) and slow (*green*) spread from the caudad to cephalad needle. Adapted with permission from Eisenach JC, Hood DD, Curry R, Shafer SL: Cephalad movement of morphine and fentanyl in humans after intrathecal injection. ANESTHESIOLOGY 2003; 99:166–73.

Pharmacology of Spinal Analgesia

Funding during this period was based on a National Institutes of Health grant (No. R37GM48085) that is still active after nearly 30 yr, with Alison Cole, Ph.D., as my program director. Alison retired in 2020, and the ASA and Foundation for Anesthesia Education and Research thanked her in person for her service to many anesthesiologist–scientists at presentations of the 2021 ASA Annual Meeting. The goals of these studies were to test whether drugs producing analgesia in animals also worked in humans. These studies paralleled or followed those of Swedish physicians including a Ralph Waters trainee and longtime friend of John Severinghaus, Torsten Gordh, Sr., M.D., who introduced anesthesiology to Sweden and lidocaine to the world[18]; Narindar Rawal, M.D., who helped introduce morphine[19]; Torsten Gordh, Jr., M.D., who introduced clonidine[20]; and Alf Sollevi, M.D., who introduced adenosine[21] for neuraxial use.

The first drug we tested was built on anatomic and physiologic studies showing that neurons in the locus coeruleus project to the spinal cord, releasing norepinephrine, which causes analgesia by stimulating α2-adrenoceptors (fig. 4A). Systemic morphine activates this circuit, and it's now believed that norepinephrine reduces pain directly by an α2-adrenergic mechanism and indirectly by stimulating spinal acetylcholine release, which is also analgesic (fig. 4B). In a clinical study for another purpose, we amended the protocol to test this idea: I stayed in the research unit overnight with a spinal catheter in place and received IV morphine on the next day. Norepinephrine increased 2.5-fold and acetylcholine increased 10-fold in my CSF after IV morphine, peaking at the time of maximum effect.[22]

These and other studies led us to "wonder if…" spinal neostigmine would be analgesic in humans *via* acetylcholine-augmenting effects and if systemic opioids and epidural clonidine would be potentiated by this mechanism. To test neostigmine's effect on IV opioid analgesia, we gave alfentanil by IV computer-controlled infusion to targeted plasma concentrations and showed that the concentration response curve for analgesia was shifted up and to the left, meaning it was potentiated, when spinal neostigmine was administered.[23] To test neostigmine's effect on epidural clonidine analgesia, we gave clonidine by computer-controlled epidural infusion to targeted CSF concentrations and showed that neostigmine also potentiated clonidine.[24] Several dozen clinical trials of neuraxial neostigmine have been performed over the past 25 yr. Intrathecal injection produced analgesia in these trials, but also multiple side effects, especially severe nausea and vomiting, and its use was abandoned. Epidural injection, on the other hand, does not cause nausea and vomiting, but its use is limited to that of an adjunct to local anesthetics.

During the next 25 yr, we asked "I wonder if…" with spinal injection of other compounds, examining efficacy, side effects, pharmacokinetic/dynamic models, and mechanisms.

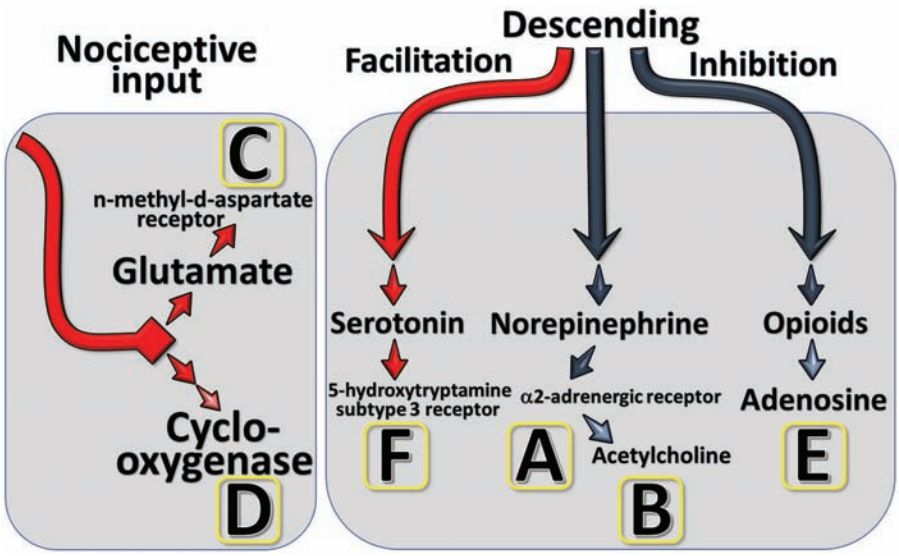


Fig. 4. Spinal pharmacology tested in humans, depicting sites of drug action in the spinal cord (*gray shaded areas*) from central terminals of nociceptive input on the left and spinal cord terminals of descending facilitation (in *red*) and inhibition (in *dark blue*). Drugs that were tested include (*A*) clonidine, an α2-adrenergic agonist that mimics norepinephrine; (*B*) neostigmine, which prolongs the action of acetylcholine released in response to α2-adrenoceptor stimulation of interneurons; (*C*) amitriptyline, which blocks *N*-methyl-D-aspartate (NMDA) receptors that are activated by glutamate release from nociceptive afferents; (*D*) ketorolac, which inhibits cyclooxygenase that is activated through processes initiated by nociceptive input; (*E*) adenosine, which is released by spinal interneurons as a result of opioid release in the spinal cord; and (*F*) ondansetron, which blocks serotonin 5-hydroxytryptamine, subtype 3 receptors that facilitate nociceptive neurotransmission.

A strong noxious stimulus releases enough glutamate into the spinal cord to stimulate *N*-methyl-D-aspartate (NMDA) receptors, which signal pain and drive sensitizing processes (fig. 4C). For this reason, we examined spinal amitriptyline, which, in addition to its monoamine reuptake properties, is an NMDA receptor antagonist. Incoming pain signals also stimulate cyclooxygenase-containing cells in the spinal cord, which release products that drive spinal sensitization (fig. 4D). For this we reason, we examined spinal ketorolac to block spinal cyclooxygenase. Some descending pain inhibitory systems release enkephalins and other opioids, which directly stimulate opioid receptors and indirectly stimulate adenosine release for analgesia (fig. 4E). For this reason, we examined spinal adenosine. Finally, painful input results not only in activation of descending systems to the spinal cord that block pain, but also some that augment pain and sensitization. The clearest example from animal work is serotonin (fig. 4F). For this reason, we examined spinal ondansetron to block serotonin receptors and stop pain amplification.

Is this kind of work gadgeteering or creation? Severinghaus had this to say: "An aha! is a sudden insight into something that you hadn't seen connect before. […] The nature of genius perhaps, or of creation, is when our mind suddenly connects two things that had no connection before in a way that's creative and new and makes something work that didn't work before. Now, I didn't have an 'aha!', I just took their devices and worked with them and built them."[3] Perhaps he's too hard on himself, but I, too, felt that my work during this time might be useful, but was not particularly creative.

Table 1 shows the steps we used for testing: replication (Could we replicate an analgesic effect in animals in our own laboratory?); toxicology (Is the drug toxic to the spinal cord?); efficacy; side effects in the analgesic range; and clinical utility as the balance of efficacy and side effects. For clonidine, there is utility in the restricted population of intractable cancer pain. For neostigmine, there is no utility spinally (shown in red), but there is some epidurally (in green). Notice that only two of the other four made it to human studies, and neither of those showed clinical utility: adenosine, which had low efficacy in only one type of pain; and ketorolac, which had no efficacy in any type of pain. Was this worth 30 yr of work? Spinal clonidine and perhaps adenosine have a place in treating intractable cancer or chronic pain, and our studies with neostigmine and adenosine did spur development of cholinergic and purinergic receptor agonists and modulators, so, maybe yes. The two drugs that did not make it to humans also advanced our understanding.

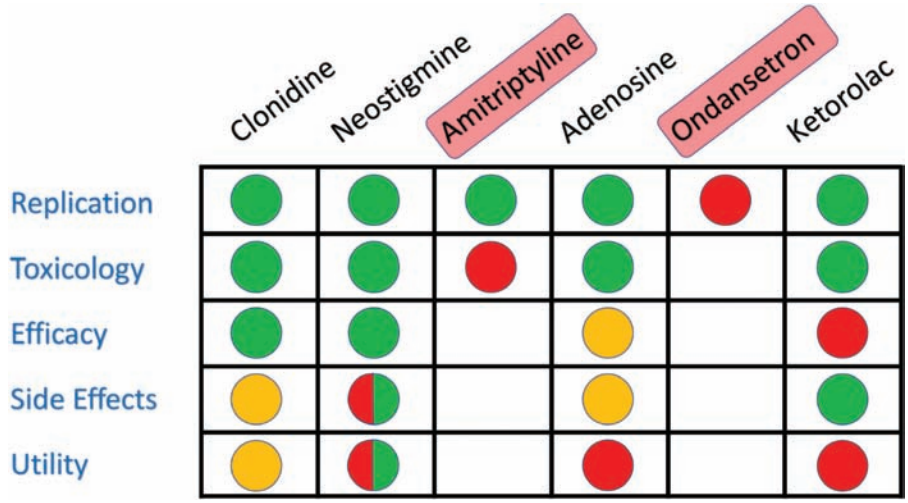
First, in a multicenter study with Tony Yaksh, we failed to replicate analgesia from spinal ondansetron in rats.[25] There was a growing realization at this time that many findings in basic research in prestigious journals could not be replicated, and that this reflected very low scientific quality. John Ioannidis stated that these research findings may often be simply accurate measures of the prevailing bias,[26] and I wrote an editorial showing this to be the case with studies of low scientific rigor I myself had published early in my career.[27] An article that applied principles of evolution to this concern noted that "…an incentive structure that rewards publication quantity…will lead to the natural selection of poor methods and increasingly high false discovery rates."[28] The reasons for this are many and are entrenched in basic science culture. At Anesthesiology, we responded with a policy to ensure that authors at least report basic aspects of study design, such as blinding and randomization, but we have far to go to fix this problem.[29]

Second, we showed neurotoxicity from spinal amitriptyline in rats that had been missed in previous behavioral studies.[30] Tony Yaksh has studied safety of spinally administered drugs for his entire career and advised the Food and Drug Administration on spinal drug development. He often quotes a founder of pharmacology, Paracelsus, who stated in 1532, "All things are poisons. It is only the dose which makes a thing poisonous." Dose was the reason women were paralyzed by accidental spinal injection of 2-chloroprocaine intended to be epidural.[5] Tony also showed that concentration and time of exposure explained paralysis in patients when lidocaine was slowly infused through a spinal microcatheter.[31] He showed that all NMDA antagonists are spinally toxic in dogs[30]; others observed similar toxicity from spinal ketamine in people.[32] At Anesthesiology, we adopted a policy not to publish clinical studies of neuraxial or perineural injection of drugs not Food and Drug Administration–approved for those routes unless there was Food and Drug Administration oversight, or there was toxicity screening in the literature, or there was a long history of use (see the journal's online Instructions for Authors). We applied this policy as an example of the central ethical concept in medicine to first do no harm. This policy has now been adopted by many journals in our specialty and beyond.

Toward the end of Act II, I, like Severinghaus, was asking broader questions that required entire teams. The National Institutes of Health supports team science with large grants, and after 2 yr of effort, we secured funding for a team of chronic pain clinicians, physiologists, receptor biologists, and neuroscientists, supported by an external advisory board of national pain research leaders to study what we called "Pharmacologic Plasticity in the Presence of Pain" (National Institutes of Health award No. P01-NS41386). We asked why some drugs like opioids lose efficacy with chronic treatment whereas others, like clonidine, show better efficacy for chronic than acute pain.

My own laboratory was at its largest at this time, with fellows who would achieve leading research positions in their home countries of France, Japan, Korea, the United Kingdom, and Hungary, or who would immigrate to the United States to leading positions. I told new fellows in the laboratory at the time that it was a teaching laboratory,

Table 1. Testing Steps and Results in Clinical Studies of Spinal Analgesics



Each drug was first tested in animals for replication of work published by others and for toxicology when administered intrathecally in rats and dogs, then was tested in small clinical studies for efficacy to experimental and clinical pain, side effects, and overall utility. *Green* and *red* indicate success and failure at each stage, with *yellow* indicating only partial success. Amitriptyline and ondansetron (*highlighted*) were halted for toxicity or failure to replicate efficacy in animals, respectively. Neostigmine showed good clinical utility with epidural administration but severe side effects and resultant poor utility with intrathecal administration.

that education was its primary purpose, and that science happened as a byproduct. Looking back on this time, I was kidding myself in that I was learning from them as much as I was teaching. As Joseph Campbell states, "Just as the people who you met by chance became effective agents in the structuring of your life, so you have been an agent in the structuring of other lives."[2] This was also true in my work at Anesthesiology, where I taught and learned from people at different stages of journeys within and outside our specialty. Perhaps the greatest honor I have received in my work life was to give the Rovenstine lecture in 2015,[33] and the audience and I learned so much more by including Anesthesiology Associate Editor Carol Cassella in the creation and performance of that lecture.

This period was very unsettling to me, and perhaps to Severinghaus, as he thought about his electrodes. "I'm afraid I have to admit that that's what everybody remembers me for. And it probably will be, if anything, it goes down in history as being my creation. That doesn't make me particularly happy that that is it, because I would have liked to think that some of my physiologic ideas have been more important. I'm having more trouble selling that notion."[3] During this time, I felt that the goal became less clear, the path more obscure, and the meaning less certain. Some of our clearest findings, such as toxicity (which had been missed by others), lack of replication, and lack of efficacy in humans, seemed to have no impact on the entrenched culture that primarily valued new targets, new circuits, and new mechanisms. I also wondered whether my work might actually cause harm, like previous thought leaders who encouraged deadly practices like epidural injection of 150 mg bupivacaine over 2 min for cesarean delivery, or 10 mg epidural morphine for postoperative analgesia with only 30 min of respiratory monitoring. I have since come to terms with this risk of harm, as all scientists must, but nearly left research altogether during this period. Two mentors, colleagues, and friends, Douglas Ririe, M.D., Ph.D., and Timothy Houle, Ph.D., listened, encouraged, and supported me in finding my path forward through Act II.

Act III: I Wonder Why?

This brings us to Act III, the return, in which the physician–scientist asks, "I wonder why…?" As Severinghaus said, it's about hypotheses: "I think that a paper which not only does research and reports it, but creates a hypothesis which is testable and stimulates other people is perhaps the most important thing you can do. And that's what I really would like to be remembered for."[3] Clearly he is remembered for this. Dr. Beverley Orser, the 2020 Severinghaus Lecturer, mentioned to me that she witnessed firsthand the tremendous gift John had to ask himself and others the right questions to generate those kinds of studies.

When asked about the future of arterial blood gas analysis, John Severinghaus said, "There is a fiber optic catheter that can be slipped in through a 20-gauge needle into an artery and give you pO_2, pCO_2, and blood pressure. Now, having said that, I don't think it's going to be a big success because it's invasive."[3] I moved away from invasive spinal injections for the same reason.

The question I'm now asking is about recovery from pain after surgery. We need to start thinking about this in a different way. When our daughter, Laurel, was in labor with her first child, I texted Dr. Pamela Flood, who had

created new mathematical models for labor progression. She sent me a link, where I entered Laurel's age, weight, race, and gestation, and plotted each cervical dilation as reported to us by her husband. Dr. Flood's model precisely predicted Laurel's labor course. Obstetricians would scoff at not considering labor a process. Yet we have only rudimentary knowledge of the time course of recovery from pain after surgery—we just say pain is chronic or gone. To better understand this, we measured daily pain for 2 months in several hundred women after cesarean delivery; our modeling showed five patterns.[34] When Laurel later had a cesarean delivery, she sent me pain scores for a few days, and I accurately predicted the group she belonged to and her time to pain resolution.

There are other pain syndromes where we do consider recovery as a process. A few years ago my father self-diagnosed a painful rash in a low cervical dermatome as shingles, even though he had received the shingles vaccine. I reassured him that, like the current vaccine for COVID-19, the first-generation shingles vaccine he had received was documented to reduce the likelihood and severity of acute disease and speed recovery, and it did for him. In looking for a vaccine against chronic postsurgical pain, we need clues for the tremendous interindividual variability in patterns of recovery from pain. This is the "I wonder why…?" question.

Noradrenergic Processing

One clue is the admonition, which appears to be well founded, that you have to work through the pain to get better. This conflicts with modern pain theory that repeated acute pain, such as each step after a knee replacement, causes pain chronicity rather than recovery. To sort this out, we spent a few years gathering preliminary data and brought together a multidisciplinary team of basic and clinical researchers and an external advisory board and received a large team science grant in 2016 that ends in 2022 (National Institutes of Health award No. P01-GM113852).

The underlying hypothesis is simple (fig. 5A). Each step after knee surgery signals pain to the spinal cord and is transmitted to higher centers. Some higher centers send signals back to the spinal cord. These don't dampen each jolt of pain, but over time, they do help resolve the pain-amplifying sensitization in the spinal cord caused by surgery. In rats, postoperative sensitization is measured by a low threshold to withdrawal from touch, which recovers in about 2 to 3 months, similar to recovery time from postoperative pain in humans. If the descending norepinephrine fibers are destroyed, recovery is blocked.[35] We've replicated this finding several times, suggesting that this system is critical to recovery in rodents. We then developed a noninvasive way to quantify the transient activation of the norepinephrine system in people *via* acute pain stimulus and are testing two hypotheses in a multicenter clinical trial with Daniel Sessler, M.D.

The first is that a larger norepinephrine response to acute pain, as measured by our noninvasive test, will predict quicker recovery from pain after knee or hip arthroplasty during the course of 6 months. The second relates to the clear failure of gabapentin to speed postoperative recovery from pain. In 2021, a meta-analysis examined 110 studies in more than 19,000 patients, concluding that the effects of gabapentin were small and of uncertain clinical relevance.[36] We have repeatedly shown that gabapentin increases tonic firing in the locus coeruleus in rodents,[37] and this makes us think that we might be able to predict patients for whom gabapentin would produce a benefit. In cardiac physiology, we know from the Frank–Starling relationship that IV fluid will improve or worsen stroke volume in patients with heart failure, depending on the starting end-diastolic volume (fig. 5B). Others have shown a similar relationship between tonic activity in the locus coeruleus and its acute response to a stimulus. We hypothesize that gabapentin, by increasing tonic locus coeruleus activity, will increase the acute response with each step during recovery in some individuals, and decrease it in others, depending on the level of tonic activity before gabapentin (fig. 5C). Based on this, we are testing whether knowing each patient's tonic activity in this system before surgery will allow us to predict preoperatively who might benefit from gabapentin with quicker recovery and who might be harmed by gabapentin.

Oxytocin

The other clue comes from the observation that there is a faster recovery from pain after childbirth, including cesarean delivery, than after other major surgical procedures.[38] More rapid recovery from injury also occurs in rodents and can be reversed by blockers of oxytocin,[39] which is normally released into the blood and into the spinal cord during labor and for weeks after delivery. After several years gathering preliminary data, we formed a multidisciplinary team of investigators from Stanford to Sweden under the leadership of T. Jeff Martin, Ph.D., with advice from experts from Germany to San Francisco to test how oxytocin speeds recovery from injury in rats and to prepare for clinical trials.

Oxytocin can act peripherally and centrally to relieve pain, but we're really interested in the periphery because of a new story. Normal input from peripheral nerves, like when you're pinched, results in pain. But abnormal input, like light brushing on sunburned skinned, can also cause pain. It's assumed that peripheral nerve fibers are injured during surgery, providing abnormal input and pain, and that this resolves in a few weeks, so any pain after this must reflect abnormal central sensitization alone. In our laboratory, Mario Boada, a wizard at neural recording and the smartest physiologist I know, has shown instead that surgical injury affects both pain and touch fibers and that the time-course of their recovery exactly parallels behavioral recovery during the months[40] after surgery, meaning it's mostly abnormal input, not central changes. More excitingly, he's shown that oxytocin can reverse the abnormal peripheral input causing prolonged pain.[41]

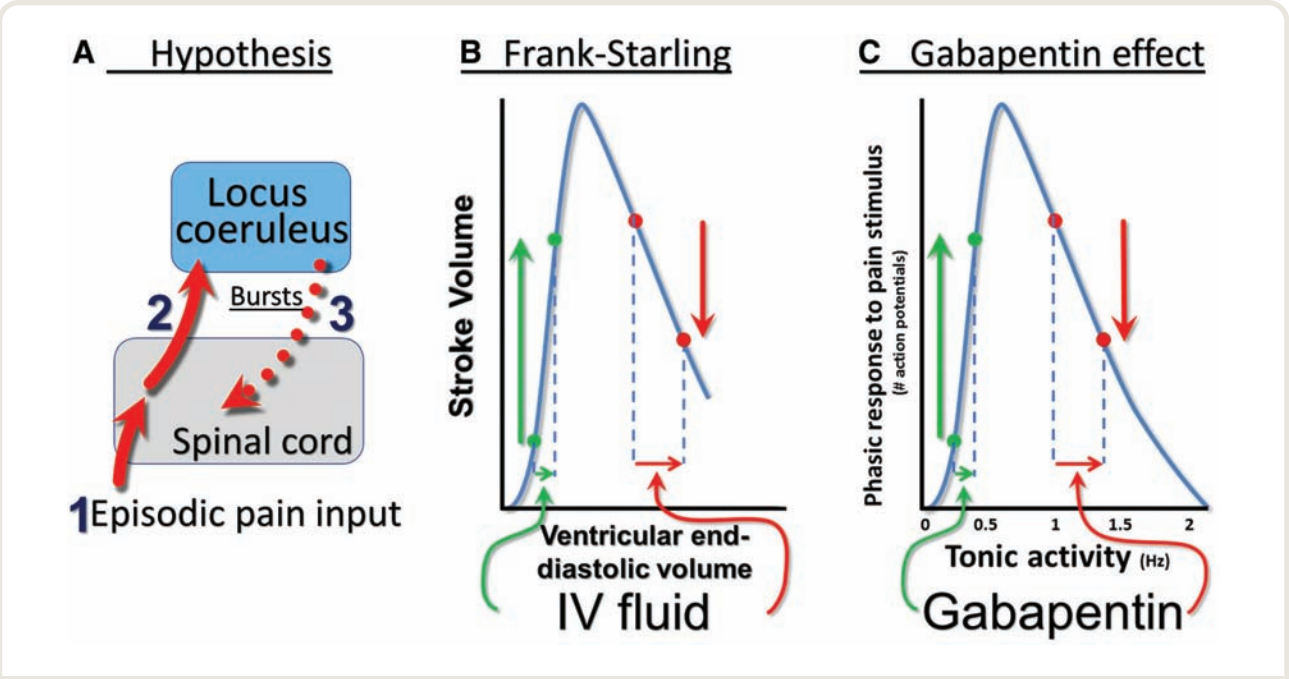


Fig. 5. Basis for a clinical trial to assess and manipulate locus coeruleus function to speed recovery after surgery. (*A*) The underlying hypothesis is that (1) episodic pain with movement after surgery enters the spinal cord and is amplified by central sensitization before (2) transmission to the locus coeruleus. The locus coeruleus responds (3) with transient bursting of activity in descending projections to the spinal cord, which results over weeks in resolution of central sensitization and postoperative pain. (*B*) The Frank–Starling relationship between end-diastolic volume and cardiac stroke volume in patients with heart failure. At low end-diastolic volume, shown in *green*, intravenous (IV) fluid increases stroke volume, whereas at high end-diastolic volume, shown in *red*, IV fluid exacerbates heart failure and decreases stroke volume. (*C*) Hypothesized relationship between tonic activity of the locus coeruleus and phasic response to a pain stimulus and the influence of gabapentin. At low rates of tonic locus coeruleus activity, shown in *green*, gabapentin (which increases tonic locus coeruleus activity) increases the phasic response to intermittent pain, whereas with high resting tonic locus coeruleus activity, shown in *red*, gabapentin would decrease the acute response to pain.

We know little of peripheral oxytocin pharmacodynamics outside of obstetrics, and little about pharmacokinetics due to problems with historical assays. Again, we relied on Steve Shafer's help to move us forward, determining oxytocin pharmacokinetics in blood with a better assay, developing noninvasive measures of peripheral and central oxytocin action in men and women, and creating preliminary kinetic/dynamic models after computer-controlled IV oxytocin infusions to fixed plasma concentrations. These models predict that an infusion at a fixed plasma concentration over minutes can produce primarily peripheral effects, that a brief infusion at a higher concentration can produce primarily central effects 12 h later, and that a sustained infusion at a much lower concentration over days can produce a primarily peripheral effect. We will test these predictions over the next 5 yr to allow proper design of clinical trials after surgery. We will also study intranasal oxytocin, a more practical route for prolonged treatment.

You cannot imagine the joy of this part of my journey, returning vitalized and changed, sparking creativity together with other wonderfully vital scientists. Mythical journeys are usually walked once, whereas each of us starts afresh on paths each day or year. In science, these journeys include the assertion "I will show…" and the questions "I wonder if…?" and "I wonder why…?" It's worth noting that another door opened for me at the start of this phase, and it has been my honor to serve with a remarkable team, the Board of Directors of Foundation for Anesthesia Education and Research and my joy as we go about supporting investigators as the curtain rises on their plays.

A Brief Postlude

In preparing this talk, I came across a photo of me in the laboratory office and noticed a book sitting by my shelves. It's one of Piet Hein's small books of haiku-like sayings or poems, which he called *gruk*. Hein was a Danish physicist, architect, and artist, and perhaps because he was all these things at once, John Severinghaus was drawn to him and would often quote a *gruk* in his scientific articles. At the end of announcing his good friend John Severinghaus as the recipient of the ASA Excellence in Research Award in 1986,[42] Thomas Hornbein ended with what he considered to be John's favorite *gruk*[43]:

I'd like to know
what this whole show
is all about
before it's out

I believe John did know what it was all about because he experienced it himself. Joseph Campbell, a Buddhist at heart, questioned whether there was any purpose or meaning in life, but was convinced that myths gave us clues to being truly alive as one is following their bliss. To Campbell, following one's bliss meant participating fully in life in what he called "a wonderful opera, but painful."[2] To me it's evident that John Severinghaus was following and experiencing his bliss. As I ended the lecture with a photo of John Severinghaus and his wry smile, I could almost hear him repeating the words of Campbell: "If you do follow your bliss you put yourself on a kind of track that has been there all the while waiting for you, and the life that you ought to be living is the one you're living. I say follow your bliss and don't be afraid, and doors will open where you didn't know they were going to be."[2] Like each of us, John Severinghaus had many doors opened for him, just as he opened many for others. He would—as I do—want each of us to go out and open doors for others.

Acknowledgments

The author dedicates this lecture to his father, John Eisenach, M.D., a contemporary of John Severinghaus who attended the lecture virtually from his home, and who has inspired the author as a World War II veteran, a family doctor and then anesthesiologist, a pilot, a singer in retirement, and an exemplary and loving father to this day. The journey of a physician–scientist's career reflects many helping hands, visible and invisible. The author notes that, if along the way he has listened to better understand others, if he has been kind or generous along this journey, it's because of the love and example of his mother, Minnie, and wife, Patricia.

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Competing Interests

Dr. Eisenach is President of the Foundation for Anesthesia Education and Research, an organization related to the American Society of Anesthesiologists (Schaumburg, Illinois). Dr. Eisenach holds U.S. patents 60/356,280 ("Compositions and Methods for Treating Pain Using Cyclooxygenase-1 Inhibitors") and 6,248,744 ("Method for the Treatment of Female-specific Pain") related to the content of this article. Products have not been, nor are currently, in development under these patents.

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ANESTHESIOLOGY

Quantitative Neuromuscular Monitoring in Clinical Practice: A Professional Practice Change Initiative

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Quantitative (train-of-four ratio) monitoring is the definitive standard for assessing recovery from neuromuscular block
- Residual neuromuscular block, defined as a train-of-four ratio less than 0.9, is commonly observed in patients given nondepolarizing neuromuscular blocking drugs perioperatively
- Inadequate reversal of residual neuromuscular block is associated with postoperative morbidity and mortality
- Despite guidelines from several professional societies advocating quantitative neuromuscular monitoring for neuromuscular blocking drug management, it is infrequently used

What This Article Tells Us That Is New

- A departmental professional practice initiative began with the goal of documenting a train-of-four ratio greater than or equal to 0.90 for all patients given a nondepolarizing neuromuscular blocking drug
- This retrospective assessment of the implementation of documenting train-of-four ratios greater than or equal to 0.9 before extubation improved from 1% (2 of 172) of cases in November 2016 to 93% (250 of 269) of cases in December 2020
- Attaining this endpoint required not only placing a quantitative monitor in each anesthetizing location but also ongoing educational efforts and follow-up

ABSTRACT

Background: Residual neuromuscular blockade can be avoided with quantitative neuromuscular monitoring. The authors embarked on a professional practice initiative to attain documented train-of-four ratios greater than or equal to 0.90 in all patients for improved patient outcomes through reducing residual paralysis.

Methods: The authors utilized equipment trials, educational videos, quantitative monitors in all anesthetizing locations, and electronic clinical decision support with real-time alerts, and initiated an ongoing professional practice metric. This was a retrospective assessment (2016 to 2020) of train-of-four ratios greater than or equal to 0.9 that were documented before extubation. Anesthesia records were manually reviewed for neuromuscular blockade management details. Medical charts of surgical patients who received a neuromuscular blocking drug were electronically searched for patient characteristics and outcomes.

Results: From pre- to postimplementation, more patients were assigned American Society of Anesthesiologists Physical Status III to V, fewer were inpatients, the rocuronium average dose was higher, and more patients had a prereversal train-of-four count less than 4. Manually reviewed anesthesia records (n = 2,807) had 2 of 172 (1%) cases with documentation of train-of-four ratios greater than or equal to 0.90 in November 2016, which was fewer than the cases in December 2020 (250 of 269 [93%]). Postimplementation (February 1, 2020, to December 31, 2020), sugammadex (650 of 935 [70%]), neostigmine (195 of 935 [21%]), and no reversal (90 of 935 [10%]) were used to attain train-of-four ratios greater than or equal to 0.90 in 856 of 935 (92%) of patients. In the electronically searched medical charts (n = 20,181), postimplementation inpatients had shorter postanesthesia care unit lengths of stay (7% difference; median [in min] [25th, 75th interquartile range], 73 [55, 102] to 68 [49, 95]; $P < 0.001$), pulmonary complications were less (43% difference; 94 of 4,138 [2.3%] to 23 of 1,817 [1.3%]; $P = 0.010$; –1.0% difference [95% CI, –1.7 to –0.3%]), and hospital length of stay was shorter (median [in days] [25th, 75th], 3 [2, 5] to 2 [1, 4]; $P < 0.001$).

Conclusions: In this professional practice initiative, documentation of train-of-four ratios greater than or equal to 0.90 occurred for 93% of patients in a busy clinical practice. Return-of-strength documentation is an intermediate outcome, and only one of many factors contributing to patient outcomes.

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The dangers of paralyzing a patient with neuromuscular blocking drugs are well recognized. Despite advances in anesthetic management, approximately half of all patients arriving to the postanesthesia care unit (PACU) suffer from residual blockade defined as a train-of-four ratio less than 0.9.[1]

Clinicians employ various methods, most of which have poor sensitivity, to determine if a neuromuscular blocking drug effect has regressed.[2,3] Using time from drug

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administration is unreliable because of the variable duration of blockade between patients for all neuromuscular blocking drugs. For example, a single dose of rocuronium may take more than 2 h to resolve.[4] Unfortunately, physical examination maneuvers such as head lift and hand squeeze lack the sensitivity and specificity needed to identify residual blockade.[5,6] Train-of-four peripheral nerve stimulation with subjective no-fade assessment is also limited as it only verifies that the train-of-four ratio has reached 0.4. Even when subjective monitoring was optimized in a research setting, residual blockade occurred in more than one third of patients.[7]

Residual blockade may contribute to a longer length of stay in the PACU,[8] more oxygen requirements in the PACU,[9] airway collapse,[10] airway obstruction,[11] severe hypoxia in the PACU, and lower quality of recovery.[12] Additionally, the use of neuromuscular blocking drugs and presumed inadequate reversal has been associated with postoperative hypoxia and desaturation, reintubation and unplanned admission to the intensive care unit (ICU),[13] pulmonary complications,[14,15] longer PACU and hospital lengths of stay,[16] and increased mortality.[17]

Anesthesiologists philosophically place emphasis on patient safety, which includes education and advocacy from professional societies, to fully rescue patients from their state of paralysis before awakening. The U.S. Food and Drug Administration (Silver Spring, Maryland) has approved devices to measure train-of-four ratios (quantitative monitoring) that provide reproducible and accurate measurements of paralysis. The cutoff used in clinical studies is a train-of-four ratio at the adductor pollicis of 0.9.[5] One study demonstrated marginal success in reducing residual paralysis by incorporating quantitative monitors into practice. In that study, PACU intubations were also reduced when appropriate monitoring was used.[18,19]

Our specific aim with this project was to develop a practice in which all patients given a nondepolarizing neuromuscular blocking drug were fully reversed from their state of paralysis as confirmed by a quantitative monitor measurement (*i.e.,* train-of-four ratio greater than or equal to 0.9). A train-of-four ratio greater than or equal to 0.9 could be attained spontaneously or facilitated with sugammadex or neostigmine. We embarked on a departmental professional practice initiative with the goal of documented train-of-four ratios greater than or equal to 0.90 for all patients. We aimed to improve patient outcomes through the subsequent reduction in postoperative residual paralysis.

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Materials and Methods

This project was performed as part of a professional practice initiative with a waiver from the Virginia Mason Medical Center (Seattle, Washington) Institutional Review Board. Written informed consent was not required from study participants. Virginia Mason Medical Center is a 300-bed urban hospital with 28 anesthetizing locations. The Department of Anesthesiology consists of 38 anesthesiologists, 27 anesthesia residents, 5 anesthesia fellows, 30 nurse anesthetists, and 19 anesthesia technicians. We utilized the SQUIRE 2.0 (Standards for Quality Improvement Reporting Excellence) quality improvement reporting guidelines.

Our department has a tradition of evidence-based protocol–driven anesthesia management, and neuromuscular blocking drug reversal protocols were in place throughout this process. The interventions of this project included educational efforts, formal trials of monitoring equipment, equipment cost assessments, placement of quantitative monitors in all rooms, instructional video production and distribution, performance feedback, and real-time automated anesthesia charting reminders.

Preimplementation

In 2016, every operating room contained a peripheral nerve stimulator (DigiStim II [Neuro Technologies, CCR Medical, Inc., USA] and EZ Stim II [Lifetech International, USA]). Optional neuromuscular monitors available in 2016 included uniaxial acceleromyography technology, namely the TOF-Watch SX (Organon, Ireland) and IntelliVue NMT (Philips, The Netherlands). Triaxial acceleromyography technology, the Stimpod 450× monitor (Xavant Technologies, South Africa), was introduced in July 2017. Electromyography technology, the TwitchView monitor (Blink Device Company, USA), was introduced in July 2018. Table 1 summarizes when equipment was available. Neuromuscular blocker management guidelines resided on the departmental website. The study period began in November 2016, and a 1995 guideline, updated in April 2016, was available for reference (Supplemental Digital Content 1 fig. 1, http://links.lww.com/ALN/C810). This guideline identified quantitative monitoring as the accepted standard for reversal assessment, but only provided reversal guidance using peripheral nerve stimulator–aided subjective assessment at the adductor pollicis. Clinical signs alone (*e.g.*, 5-s head lift) were identified as unreliable. Neostigmine reversal at train-of-four counts 1 and 2 was allowed using higher neostigmine doses (50 and 60 mcg/kg, respectively) but waiting for further recovery was recommended before administration of the reversal drugs. Neostigmine reversal

(50 mcg/kg) at train-of-four count 3 was allowed, but again, waiting was recommended. Reversal at train-of-four count 4 was identified as best practice, with recommendations of 40 mcg/kg neostigmine with fade and 20 mcg/kg without fade. An updated 2018 guideline differed; it offered specifics regarding quantitative monitoring and sugammadex (Supplemental Digital Content 2 fig. 2, http://links.lww.com/ALN/C811). Acceleromyography train-of-four ratios greater than or equal to 1 and electromyography train-of-four ratios greater than or equal to 0.9 were recommended before extubation. If these criteria were met spontaneously, then no reversal was recommended. Neostigmine reversal was only recommended at train-of-four count 4; at all other train-of-four counts, it was recommended to wait or use sugammadex per manufacturer dose recommendations. Choice of reversal medication was left to provider discretion for train-of-four count 4, although we encouraged neostigmine when there was sufficient time for recovery (10 to 20 min).

Implementation of Quantitative Monitoring

In 2018, new departmental leadership approved the necessary equipment purchases to initiate this project. On October 4, 2019, quantitative monitors were placed in all anesthetizing locations. Monitor selection was driven by in-house trials, formal feedback from users at our institution, informal review, and cost considerations. We originally sought a single monitor solution to streamline cost and education, but enthusiasm for both monitors changed our focus. We produced cost projections for 28 anesthetizing locations based on quotes received. Although the Stimpod ($1,250) acquisition cost was lower than the TwitchView ($1,995), our purchase decision was driven by projected disposable costs. TwitchView disposable arrays ($20 to $25 each) contrasted with readily available, low-cost electrocardiogram patches used with the Stimpod. We used historic case volume data specific to each operating room to estimate the yearly array costs when preferentially placing TwitchViews in rooms with tucked cases. We presented the department with array cost projections to support three and five TwitchViews ($28,800/yr and $48,000/yr, respectively). The department wanted these monitors preferentially for tucked cases, so we purchased four TwitchViews that were to reside in rooms with more tucked cases (*e.g.*, robotic rooms, cardiac rooms). Stimpods were placed in the other 24 locations. Extra monitors of each type were available upon request to allow for provider preference.

Education, Reminders, and Performance Feedback

Short instructional videos about the use of Stimpod and TwitchView monitors were developed and made available by December 2019. The videos explained each technology (acceleromyography and electromyography), detailed how to place the electrodes on a patient, and showed the menu items of each device. Automated alerts using Smart Anesthesia Manager (Perimatics LLC, USA) were launched on June 1, 2020. Smart Anesthesia Manager is a customizable, clinical decision support system that combines preoperative and intraoperative data to provide real-time point-of-care guidance reminders. The application provides decision support reminders for a variety of clinical areas including glycemic control, nausea prophylaxis, antibiotic delivery, blood pressure management, and neuromuscular blockade reversal. For neuromuscular blockade reversal, the reminders were triggered by the charting of reversal drug administration after a nondepolarizing neuromuscular blocker. The alerts were triggered 5 min after the reversal drug to remind the provider to chart the train-of-four ratio if none was present on the record. The alert disappeared upon recognition or after 3 min. An additional alert prompted documentation of the train-of-four ratio if a patient received a nondepolarizing neuromuscular blocker, extubation was documented, and no train-of-four ratio was recorded. Alert parameters were customized based on clinician feedback to optimize provider recognition while avoiding alarm fatigue. These alerts were only a reminder to the provider, and documentation nonadherence did not impede finalization of the anesthesia record.

Performance on departmental train-of-four ratio documentation was communicated periodically to all providers beginning in June 2019. This included department-wide emails, as well as individual emails, detailing successes and

Table 1. Equipment Available from 2016 to 2020

Year	Equipment	Location/Availability
2016	DigiStim II and EZ Stim II peripheral nerve stimulators	All operating rooms
	TOF-Watch SX neuromuscular monitor	Available
	IntelliVue NMT neuromuscular monitor	Available
2017	Stimpod 450× neuromuscular monitor	Available
2018	TwitchView neuromuscular monitor	Available
2019	Stimpod 450× and TwitchView	All operating rooms

Equipment manufacturers include Neuro Technologies, CCR Medical, Inc. (DigiStim II; USA); Lifetech International (EZ Stim II; USA); Organon (TOF-Watch SX; Ireland); Philips (IntelliVue NMT; The Netherlands), Xavant Technologies, (Stimpod 450×; South Africa), and Blink Device Company (TwitchView monitor; USA).

opportunities for improvement. In January 2020, departmental leadership announced to the department that train-of-four ratio documentation would become an Ongoing Professional Practice Evaluation metric starting in July 2020. Our hospital credentialing committee subsequently required train-of-four ratio documentation in at least 70% of cases. Two providers that fell below this threshold were informed of the need for corrective action.

Manually Reviewed Anesthesia Records: Implementation Tracking

Anesthesia records were manually reviewed to track the implementation of quantitative monitoring over time. Manual anesthesia record review was a labor-intensive effort limited by personnel availability. We estimated each record required 2 to 5 min to review. Cases with missing information required more time to check multiple monitoring and comment fields before concluding the information was not present. We initially anticipated two sets of data per year for nine time points. We balanced the time requirement with the need for sufficient data to come to the decision of assessing 2 weeks of cases—approximately 200 cases—for each time point, which would total 1,800 cases and take between 60 and 150 h for our anesthesia record data reviewers. The first records reviewed were from November 2016, starting 1 month after our adoption of electronic anesthesia charting. June and November records were reviewed in 2017 and 2018. June and October records were reviewed in 2019 because October 4, 2019, was the date at which all anesthetizing locations were outfitted with quantitative monitors. January, March, June, September, and December records were reviewed in 2020 to more closely track the rapidly changing practice after quantitative monitors were available for all cases (fig. 1). The anesthesia record review process began with electronic identification of records containing rocuronium or cisatracurium, the two nondepolarizing neuromuscular blockers used at our institution. Cases with a defasciculating dose of rocuronium (less than 10 mg) before succinylcholine were eliminated. Extubation was verified in the emergence notes. Data collected included weight, neuromuscular blocker total dose, reversal drug total dose, train-of-four count before reversal, and assessment for return of muscle strength. Return of muscle strength assessments included physical signs alone, peripheral nerve stimulation–assisted subjective evaluation, and quantitative assessment. In anesthesia records with more than one assessment technique documented, the most sensitive technique, associated with the highest train-of-four ratio, was recorded. Considered to be most sensitive was 100-Hz tetany without fade (train-of-four ratio greater than or equal to 85%), followed by double-burst stimulation without fade (train-of-four ratio greater than or equal to 60%), train-of-four without fade (train-of-four ratio greater than or equal to 40%), 50-Hz tetany without fade (train-of-four ratio greater than or equal to 40%), and train-of-four count alone.[5] Any assessment without fade was considered more sensitive than assessments recognizing fade. Only in the absence of any other assessments were physical signs alone recorded. Recorded physical signs included 5-s head lift and 5-s arm lift that occurred three times (5-s head lift in June 2017 and October 2019, and 5-s arm lift in June 2018). Our aim in this professional practice initiative was to convert from subjective measurements to quantitative measurement, so we report all subjective measurements collectively.

Electronic Medical Chart Data Set: Generalized Surgical Population Outcomes

We also electronically searched medical charts for all patients receiving a neuromuscular blocking drug from January 2016 to December 2020 to assess outcomes in this retrospective observational study. All charts from our datamart with a medication order of rocuronium, cisatracurium, neostigmine, or sugammadex were eligible. Charts were deleted for patients younger than 18 yr. Use of a nondepolarizing neuromuscular blocking drug solely for defasciculation before succinylcholine could not be determined in the electronic chart search, so rocuronium doses less than 10 mg were eliminated. Within the electronic search capabilities, there was no reliable way to differentiate between patients extubated in the operating room and transported to the ICU from those who remained intubated upon transport to the ICU. We explored search strategies (*e.g.,* PACU stay before transfer to ICU) but found we could not accurately perform that search. Therefore, patients with an ICU stay at any point during hospitalization (indicated *via* presence of an ICU note) or those undergoing a cardiac procedure were excluded from the electronic medical chart data set. For patients with two surgeries in one day, only the data from the first surgery were analyzed. PACU length of stay was determined using PACU discharge-ready as well as PACU discharge. PACU discharge-ready criteria in our institution included Aldrete score 8 to 10; patent airway; oxygen saturation greater than 92% on room air or with supplemental oxygen; hemodynamic stability without requirement of vasopressors unless transferring to ICU; temperature higher than 36°C; patient orientation or baseline mental status; block level T8 or below and receding; motor/sensory function returning; pain adequately controlled and/or returned to baseline pain level; absence of active emesis; absence of bladder retention; stable surgical site/dressing; and patency of all lines. PACU lengths of stay greater than 720 min were excluded from analysis. Postoperative pulmonary complications were defined by International Classification of Diseases, Tenth Revision discharge codes consistent with previous residual blockade research[13,20] (Supplemental Digital Content 3 table 1, http://links.lww.com/ALN/C812). Hospital length of stay, discharge disposition, and readmission data were also collected.

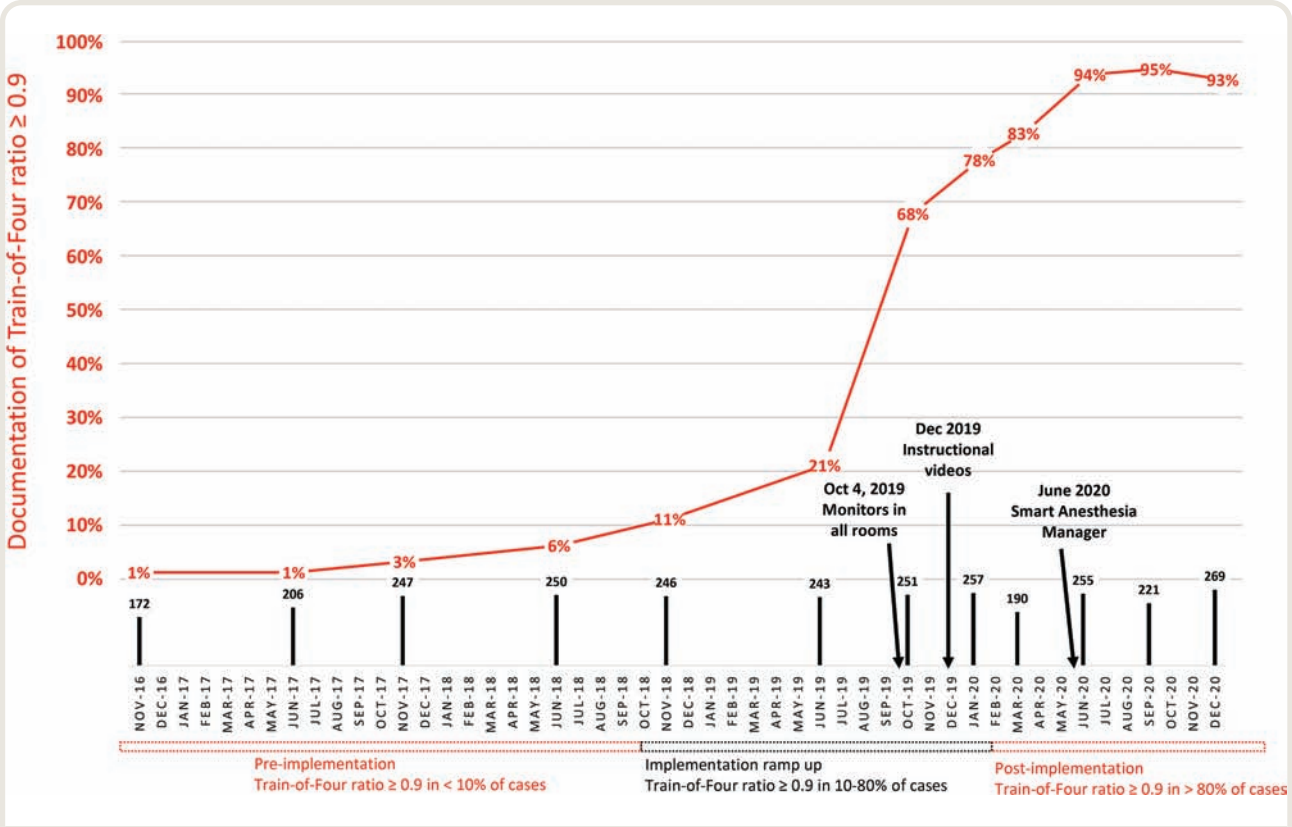

Fig. 1. Documentation trends of train-of-four ratios greater than or equal to 0.9 from 2016 to 2020 divided into three phases: preimplementation (less than 10% of cases had documentation of train-of-four ratios greater than or equal to 0.9); implementation ramp-up (10 to 80% of cases had documentation of train-of-four ratios greater than or equal to 0.9); and postimplementation (more than 80% of cases had documentation of train-of-four ratios greater than or equal to 0.9). The *red line* and numbers represent the percentage of cases documentation of train-of-four ratios greater than or equal to 0.9 on the anesthesia record. The *black bars* and numbers represent how many records were manually reviewed in each 2-week time frame.

Manual Anesthesia Record and Electronic Medical Chart Data Set Differences

The two sets of data differ fundamentally due to the ability to extract some data exclusively found in the anesthesia record. The primary purpose of the manual anesthesia record review was to track the implementation of our professional practice initiative by establishing a baseline train-of-four ratio documentation rate, and then documenting changes over time. Because of the limited number of records in this manual search strategy, we did not anticipate adequate data to assess infrequent patient outcomes (*e.g.,* pulmonary complications). To better capture patient outcomes, we conducted the electronic chart search of a larger cohort of surgical patients that were administered a neuromuscular blocker during the same period. The value of this electronic search was predicated on the assumption that train-of-four ratio documentation occurred similarly between the smaller data set of manually reviewed anesthesia records and the larger data set of electronically searched charts. An important difference between the data sets was that the manually reviewed anesthesia records included patients extubated at the end of surgery and then transferred to the ICU. Patients transferred to the ICU intubated were identifiable in the manual anesthesia records search and removed. The electronic chart data set contained no ICU patients.

Statistical Analysis

Statistical power analysis was not used to guide the sample size in this observational convenience sample. The sample size for the manually reviewed anesthesia records was limited by the availability of the anesthesiologist reviewers. The *t* test was used to compare means of continuous variables, and the chi-square to compare proportions before and after the intervention. The nonparametric Mann–Whitney U test was used to compare the medians of continuous variables that were not normally distributed. Statistical analysis was performed using StataMP 16 (Stata Corp., USA). The main analysis compared values before and after the intervention. All *P* values were two-tailed, with statistical

significance defined at $P < 0.05$. Patient and surgery characteristics were reported as number (percentage), mean ± SD, or median and interquartile range, if nonnormally distributed. The percentage change was determined by the postintervention to preintervention difference divided by the preintervention value. We acknowledge that multiple comparisons can result in falsely significant results. We provided the actual *P* values so that the reader can judge the likelihood of spurious conclusions. It is noteworthy that many of the associations would be considered significant even under conservative adjustment for multiple comparisons, such as the Bonferroni. The data analysis and statistical plan was written after the data were accessed. Ideally, we would use interrupted time series to analyze these data and adjust for known confounders. However, our intervention did not occur at a single time point, but rather was phased in, and we did not have a way to model that in a time series regression that adjusts for covariates. We chose a simple pre-/post strategy to describe the observed differences in the sample across time.

Results

The implementation graph shows the progressive increase in the percentage of cases with a documented train-of-four ratio greater than or equal to 0.9 from 2016 to 2020 (fig. 1). Manually reviewed anesthesia records (n = 2,807) showed 2 of 172 (1%) cases contained documented train-of-four ratios greater than or equal to 0.9 in November 2016 compared with 26 of 246 (11%) cases by November 2018. The introduction of quantitative monitors in all operating rooms on October 4, 2019, was associated with the greatest incremental change in documentation, from 50 of 243 (21%) in June 2019 to 170 of 251 (68%) in October 2019. In December 2020, 250 of 269 (93%) of cases contained documentation of a train-of-four ratio greater than or equal to 0.9.

Implementation of the documentation of train-of-four ratios greater than or equal to 0.9 was temporally separated into preimplementation (documentation in fewer than 10% of cases [October 1, 2016, to September 30, 2018]), implementation ramp-up (documentation in 10 to 80% of cases [October 1, 2018, to January 31, 2020]), and postimplementation (documentation in more than 80% of cases [February 1, 2020, to December 31, 2020]). This demarcation provides a comparison between subjective assessment (no quantitative monitoring, preimplementation) and quantitative monitoring (postimplementation). We manually reviewed 2,807 anesthesia records: 875 preimplementation, 997 implementation ramp-up, and 935 postimplementation. We electronically searched 20,181 medical charts: 9,034 preimplementation, 6,990 implementation ramp-up, and 4,157 postimplementation. The electronic chart search flow diagram is shown in figure 2. Our data include information unique to the manually reviewed anesthesia records data set (n = 538), information unique to the electronic chart data set (n = 17,912), and information present in both data sets (n = 2,269), for a total of 20,181 electronic charts and 2,807 manually reviewed anesthesia records.

Preimplementation to postimplementation patient demographics, surgery characteristics, neuromuscular blocker use, reversal use, and operative information from both data sets are presented in table 2. There was no difference in patient type in either data set postimplementation. In the electronic chart data set, postimplementation there were fewer inpatients: 4,138 of 9,034 (46%) preimplementation *versus* 1,817 of 4,157 (44%) postimplementation, and a longer surgical duration, mean 125 ± 88 min preimplementation *versus* 130 ± 99 min postimplementation. There were more patients with American Society of Anesthesiologists (Schaumburg, Illinois) Physical Status III to V, rocuronium use, and sugammadex use in both data sets postimplementation. Documentation of spontaneous reversal occurred in 26 of 875 (3%) cases preimplementation and 90 of 935 (10%) cases postimplementation in the anesthesia record data set. Postimplementation (February 1, 2020, to December 31, 2020), sugammadex (650 of 935 [70%]), neostigmine (195 of 935 [21%]), and no reversal (90 of 935 [10%]) were used to attain train-of-four ratios greater than or equal to 0.90 in 856 of 935 (92%) patients in the anesthesia record data set. Figure 3 demonstrates a shift over time in reversal drug use and more spontaneous reversal documentation in the anesthesia record set. A similar change in spontaneous reversal documentation occurred in the electronic chart data set: 362 of 9,034 (4%) cases preimplementation to 413 of 4,157 (10%) cases postimplementation (table 2). In postimplementation (February 1, 2020, to December 31, 2020) cases, sugammadex (2,879 of 4,157 [69%]), neostigmine (865 of 4,157 [21%]), and no reversal (413 of 4,157 [10%]) were documented in the electronic chart data set. Postimplementation patient ages were greater, there were more gynecologic and otolaryngologic cases, and there were fewer inpatients in the electronic chart data set. Importantly, the manual anesthesia record population was not a subset of the electronic chart population. There were 523 of 2,807 (19%) patients in the manual anesthesia record data set that were eliminated from the electronic chart data set. There were more inpatients (73% *vs.* 44%) in the anesthesia record data set, which included ICU patients.

Preimplementation to postimplementation data unique to the manually reviewed anesthesia records data set are presented in table 3. There was no difference in weight; average dose of cisatracurium or neostigmine; median train-of-four count before neostigmine or sugammadex administration; or average train-of-four ratio value after neostigmine, sugammadex, or spontaneous reversal. The average rocuronium dose; median overall prereversal train-of-four count; and percentages of prereversal train-of-four counts less than 4, missing pre–train-of-four count values, postreversal

train-of-four ratios greater than or equal to 0.9, and post-reversal train-of-four ratios less than 0.9 were all greater postimplementation. Documentation of train-of-four ratios greater than or equal to 0.9 occurred in 29 of 875 (3%) of cases preimplementation and 856 of 935 (92%) of cases postimplementation. Prereversal train-of-four counts equal to 4 occurred less and the average sugammadex dose was lower postimplementation. There was more frequent subjective assessment in November 2016 (146 of 172 [85%] cases) but more frequent documentation of train-of-four ratios greater than or equal to 0.9 in December 2020 (250 of 270 [93%] cases; fig. 4).

Patient outcome data from electronically searched medical charts are shown in table 4. Postimplementation, there was a shorter time from PACU arrival to ready-for-discharge (2% overall difference; median [in min] [25th, 75th], 78 [57, 110] to 76 [54, 107]; $P < 0.001$; 7% difference for inpatients; median [in min] [25th, 75th], 73 [55, 102] to 68 [49, 95]; $P < 0.001$). Time from PACU arrival to ready for discharge was not different after neostigmine, sugammadex, or no reversal. There was a shorter time from PACU arrival to discharge (4% overall difference, median [in min] [25th, 75th], 97 [72, 136] to 93 [69, 129]; $P < 0.001$). Fewer postoperative pulmonary complications occurred overall (42% difference; 104 of 9,034 [1.2%] to 28 of 4,157 [0.7%]; $P = 0.011$; −0.5% difference [95% CI, −0.8 to −0.1%]) and for inpatients (43% difference; 94 of 4,138 [2.3%] to 23 of 1,817 [1.3%]; $P = 0.010$; difference −1.0% [95% CI, −1.7 to −0.3%]). Postimplementation inpatients remained in the hospital for fewer days (hospital median length of stay [25th, 75th], 3 [2, 5] to 2 [1, 4]; $P < 0.001$). No differences were found in mortality or hospital readmissions.

Supplemental Digital Content 4 table 2 (http://links.lww.com/ALN/C813) contains patient demographic information for the implementation ramp-up period. Supplemental Digital Content 5 table 3 (http://links.lww.com/ALN/C814) contains data specific to the anesthesia records data set for the implementation ramp-up period. Supplemental Digital Content 6 table 4 (http://links.lww.com/ALN/C815) contains electronically searched medical charts outcome data for the implementation ramp-up period. Supplemental Digital Content 7 table 5 (http://links.lww.com/ALN/C816) contains the manually searched anesthesia records patient outcome data for the

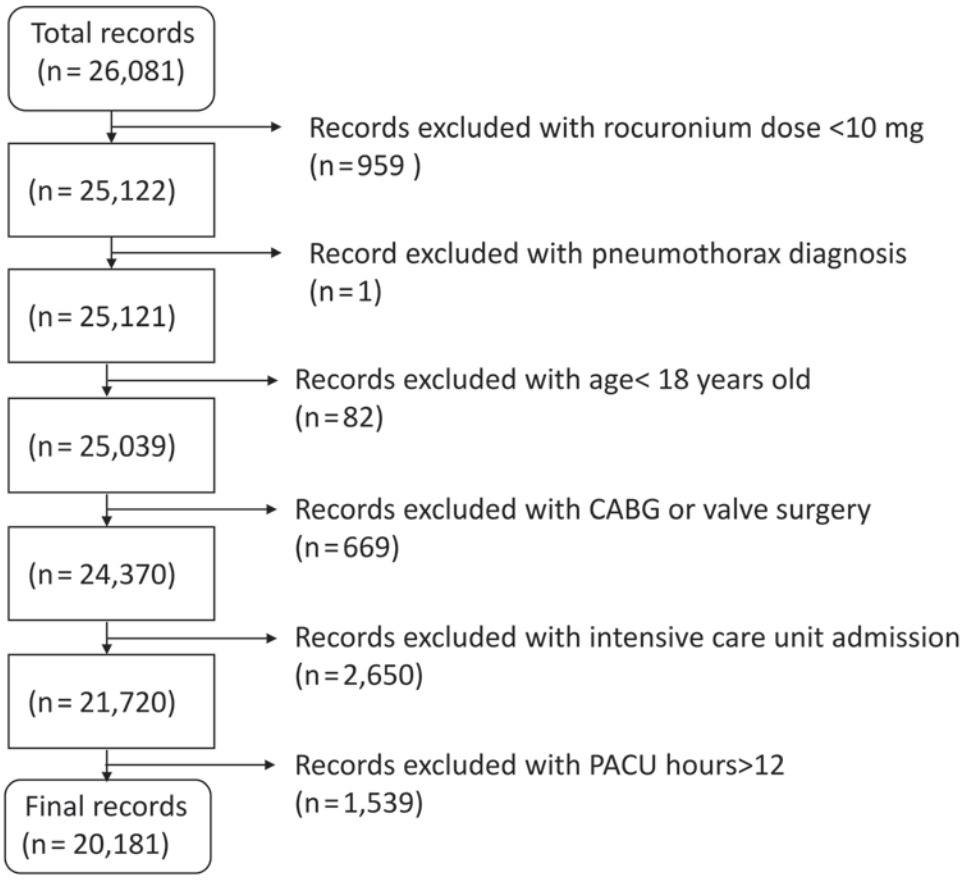

Fig. 2. Electronic chart search flow diagram showing initial surgical procedures volume, chart exclusion criteria, and final surgical procedures volume. CABG, coronary artery bypass graft; PACU, postanesthesia care unit.

Table 2. Patient Demographics, Surgery Characteristics, Neuromuscular Blocker Use, Reversal Use, and Operative Information

	Preimplementation (October 1, 2016–September 20, 2018)	Postimplementation (February 1, 2020–December 31, 2020)	*P* Value
No. patient surgeries			
Anesthesia records	875	935	
Electronic charts	9,034	4,157	
Age, yr			
Anesthesia records, mean ± SD	58.3 ± 16.1	58.0 ± 16.4	0.666
Electronic charts, median [25th, 75th]	59.3 [45.4, 69.0]	59.6 [45.4, 70.2]	0.026
Patient type, n (%)			
Anesthesia records	Female: 465 of 875 (53%); male: 410 of 875 (47%)	Female: 535 of 935 (57%); male: 400 of 935 (43%)	0.081
Electronic charts	Female: 5,176 of 9,034 (57%); male: 3,858 of 9,034 (43%)	Female: 2,428 of 4,157 (58%); male: 1,729 of 4,157 (42%)	0.228
Admit type, n (%)			
Anesthesia records			0.055
Inpatient	431 of 875 (49%)	449 of 935 (48%)	
Observation	2 of 875 (0.2%)	11 of 935 (1.2%)	
Outpatient	442 of 875 (51%)	475 of 935 (51%)	
Electronic charts			< 0.001
Inpatient	4,138 of 9,034 (46%)	1,817 of 4,157 (44%)	
Observation	25 of 9,034 (0.3%)	53 of 4,157 (1%)	
Outpatient	4,871 of 9,034 (54%)	2,287 of 4,157 (55%)	
Patient service, n (%)			
Anesthesia records			0.138
General surgery	312 of 875 (36%)	331 of 935 (35%)	
Gynecologic	74 of 875 (8%)	81 of 935 (9%)	
Otolaryngologic	94 of 875 (11%)	72 of 935 (8%)	
Other	395 of 875 (45%)	451 of 935 (48%)	
Electronic charts			< 0.001
General surgery	3,558 of 9,034 (39%)	1,620 of 4,157 (39%)	
Gynecologic	1,024 of 9,034 (11%)	361 of 4,157 (9%)	
Otolaryngologic	951 of 9,034 (11%)	342 of 4,157 (8%)	
Other	3,501 of 9,034 (39%)	1,834 of 4,157 (44%)	
ASA Physical Status class, n (%)			
Anesthesia records			< 0.001
I	90 of 875 (10%)	60 of 935 (6%)	
II	485 of 875 (55%)	457 of 935 (49%)	
III	266 of 875 (30%)	367 of 935 (39%)	
IV or V	16 of 875 (2%)	50 of 935 (5%)	
Not assigned	18 of 875 (2%)	1 of 935 (0.1%)	
Emergency	18 of 875 (2%)	35 of 935 (4%)	0.033
Electronic charts			< 0.001
I	914 of 9,034 (10%)	324 of 4,157 (8%)	
II	5,439 of 9,034 (60%)	2,190 of 4,157 (53%)	
III	2,584 of 9,034 (29%)	1,562 of 4,157 (38%)	
IV or V	97 of 9,034 (1%)	81 of 4,157 (2%)	
Not assigned	0 of 9,034 (0%)	0 of 4,157 (0%)	
Emergency	237 of 9,034 (3%)	133 of 4,157 (3%)	0.063
Admit type, n (%)			
Anesthesia records			0.936
Emergency	44 of 875 (5%)	46 of 935 (5%)	
Urgent	69 of 875 (8%)	78 of 935 (8%)	
Elective	762 of 875 (87%)	811 of 935 (87%)	
Electronic charts			0.246
Emergency	535 of 9,034 (6%)	254 of 4,157 (6%)	
Urgent	550 of 9,034 (6%)	223 of 4,157 (5%)	
Elective	7,949 of 9,034 (88%)	3,680 of 4,157 (89%)	
Neuromuscular blocking agent, n (%)			
Anesthesia records			< 0.001
Cisatracurium	93 of 875 (11%)	17 of 935 (2%)	
Rocuronium	782 of 875 (89%)	918 of 935 (98%)	
Electronic charts			< 0.001
Cisatracurium	791 of 9,034 (9%)	48 of 4,157 (1%)	
Rocuronium	8,243 of 9,034 (91%)	4,109 of 4,157 (99%)	

(*Continued*)

Table 2. (Continued)

	Preimplementation (October 1, 2016–September 20, 2018)	Postimplementation (February 1, 2020–December 31, 2020)	*P* Value
Reversal type, n (%)			
Anesthesia records			
Neostigmine	715 of 875 (82%)	195 of 935 (21%)	< 0.001
Sugammadex	134 of 875 (15%)	650 of 935 (70%)	< 0.001
No reversal	26 of 875 (3%)	90 of 935 (10%)	< 0.001
Electronic charts			
Neostigmine	7,295 of 9,034 (81%)	865 of 4,157 (21%)	< 0.001
Sugammadex	1,377 of 9,034 (15%)	2,879 of 4,157 (70%)	< 0.001
No reversal	362 of 9,034 (4%)	413 of 4,157 (10%)	< 0.001
Surgery duration, min (mean ± SD)			
Anesthesia records	141 ± 101*	139 ± 114†	0.804
Electronic charts, median [25th, 75th percentile]	125 ± 88	130 ± 99	0.004

Both data sets are presented: manual anesthesia record review (n = 2,807) and electronically searched medical charts (n = 20,181). Only the data from preimplementation and postimplementation are shown; data collected for the implementation ramp-up period are in Supplemental Digital Content 4 table 2 (http://links.lww.com/ALN/C813). Median [25th, 75th] for continuous variables; number (%) for categorical variables.

P values determined by Mann–Whitney U test for continuous variables and chi-square test for categorical variables.

*N = 843. †N = 908.

ASA, American Society of Anesthesiologists.

preimplementation, implementation ramp-up, and postimplementation periods.

Discussion

In this professional practice initiative, we accomplished documenting train-of-four ratios greater than or equal to 0.9 in the vast majority of patients. More documentation of train-of-four ratios greater than or equal to 0.90 was an intermediate outcome and one of many potential factors contributing to the observed differences in patient outcomes.

Administering sugammadex to all patients is another approach to reduce residual blockade. Sugammadex without neuromuscular monitoring appears to reduce, but not eliminate, residual blockade.[21,22] Some advocate this single-drug approach because anesthesiologists do not routinely use neuromuscular monitoring.[2,20,23,24] Sugammadex was introduced in Japan in 2010, and by 2014 was used for reversal in 95% of cases. Subsequently, concerns were raised about anaphylaxis[25] and residual blockade.[21,26] In

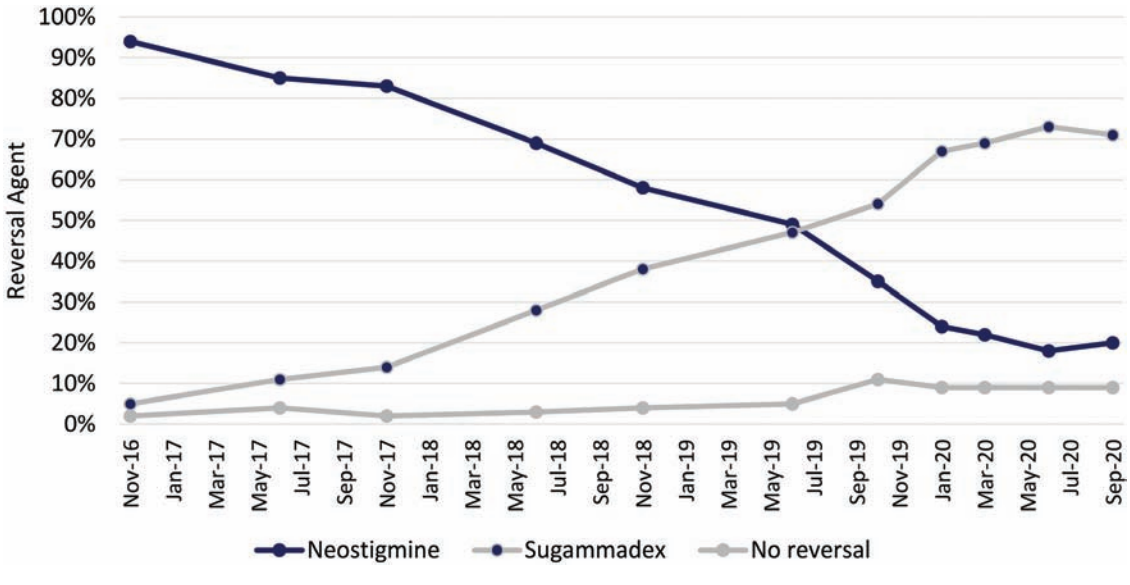

Fig. 3. Use of sugammadex, neostigmine, or no reversal drug from 2016 to 2020 in manually reviewed anesthesia records (n = 2,807).

Table 3. Neuromuscular Blocker Dose, Reversal Dose, Prereversal Assessment, and Postreversal Assessment

	Preimplementation (October 1, 2016–September 20, 2018)	Postimplementation (February 1, 2020–December 31, 2020)	*P* Value
No. patient surgeries	875	935	
Weight, kg (mean ± SD)	85 ± 23	86 ± 24	0.667
Neuromuscular blocker dose, mg (mean ± SD)			
Cisatracurium	21 ± 17	22 ± 14	0.767
Rocuronium	69 ± 41	75 ± 45	0.003
Prereversal train-of-four count, median [25th, 75th]			
Overall	4 [3, 4]*	2 [1, 4]†	< 0.001
Neostigmine	4 [4, 4]‡	4 [4, 4]§	0.118
Sugammadex	2 [1, 4]‖	2 [1, 4]#	0.542
Prereversal train-of-four count, n (%)			
Train-of-four count = 4	522 of 875 (60%)	286 of 935 (31%)	< 0.001
Train-of-four count < 4	240 of 875 (27%)	407 of 935 (44%)	< 0.001
Train-of-four count missing	113 of 875 (13%)	242 of 935 (26%)	< 0.001
Reversal dose, mg (mean ± SD)			
Neostigmine	4 ± 1	3 ± 1	0.102
Sugammadex	261 ± 123	224 ± 104	< 0.001
Operating room assessment overall, n (%)			< 0.001
Not recorded	122 of 875 (14%)	13 of 935 (1%)	
Subjective	721 of 875 (82%)	48 of 935 (5%)	
Train-of-four ratio ≥ 0.9	29 of 875 (3%)	856 of 935 (92%)	
Train-of-four ratio < 0.9	3 of 875 (0.3%)	18 of 935 (2%)	
Train-of-four ratios for train-of-four ratio ≥ 0.9 cases, mean ± SD			
Neostigmine	0.95 ± 0.14**	1.01 ± 0.15††	0.085
Sugammadex	0.95 ± 0.05‡‡	1.05 ± 0.16§§	0.059
No reversal	0.95 ± 0.07‖‖	1.07 ± 0.17##	0.328

Data are specific only to the manually collected anesthesia records data set (n = 2,807). Only preimplementation and postimplementation data are shown; data collected for the implementation ramp-up period are in Supplemental Digital Content 5 table 3 (http://links.lww.com/ALN/C814). *P* values determined by Mann–Whitney U test for continuous variables and chi-square test for categorical variables.

*N = 762. †N = 693. ‡N = 644. §N = 164. ‖N = 118. #N = 529. **N = 21. ††N = 186. ‡‡N = 9. §§N = 611. ‖‖N = 2. ##N = 77.

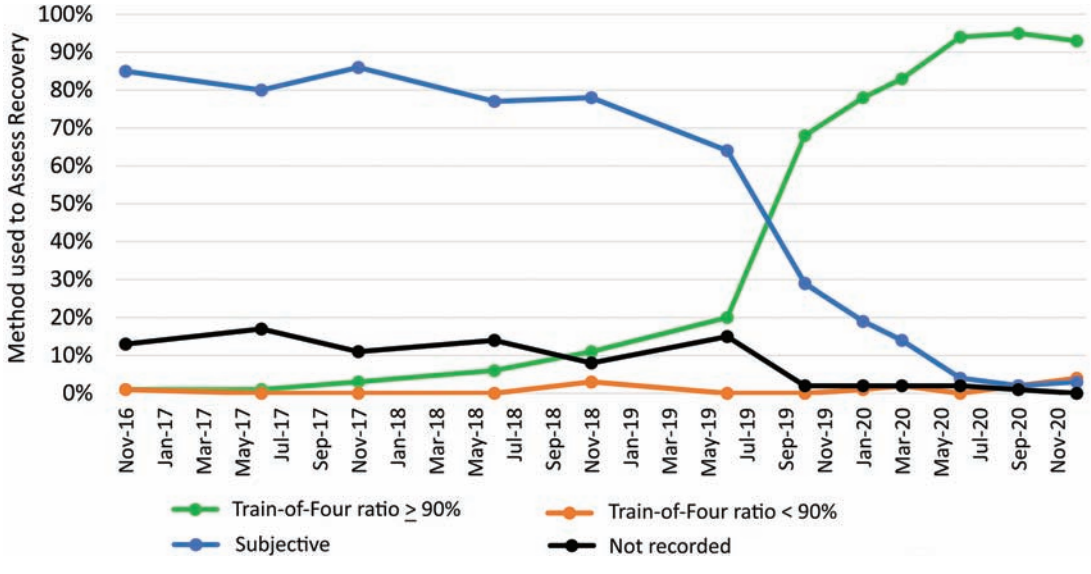

Fig. 4. Methods used to assess return of strength from 2016 to 2020 in manually reviewed anesthesia records (n = 2,807). Subjective assessment included 100-Hz tetany without fade, double-burst stimulation without fade, train-of-four without fade, 50-Hz tetany without fade, train-of-four with fade, train-of-four count 4, 5-s head lift, 5-s arm lift, and train-of-four count 0.

Table 4. Outcome Data from Electronically Searched Medical Charts (n = 20,181)

	Preimplementation (October 1, 2016–September 20, 2018)	Postimplementation (February 1, 2020–December 31, 2020)	*P* Value	Difference (95% CI)
Surgeries, n	9,034	4,157		
PACU ready for discharge by patient type, min (median [25th, 75th])				
Overall	78 [57, 110]	76 [54, 107]	< 0.001	
Inpatient	73 [55, 102]	68 [49, 95]	< 0.001	
Observation	61 [53, 83]	50 [36, 73]	0.154	
Outpatient	84 [60, 117]	85 [60, 119]	0.939	
PACU ready for discharge by reversal, min (median [25th, 75th])				
Neostigmine	79 [57, 111]	77 [56, 112]	0.438	
Sugammadex	76 [56, 107]	75 [53, 105]	0.218	
No reversal	82 [60, 117]	80 [58, 109]	0.225	
PACU to discharge, min (median [25th, 75th])	97 [72, 136]	93 [69, 129]	< 0.001	—
Postoperative pulmonary complications, n (%)				
Overall	104 of 9,034 (1.2%)	28 of 4,157 (0.7%)	0.011	–0.5% [–0.8% to –0.1%]
Inpatient	94 of 4,138 (2.3%)	23 of 1,817 (1.3%)	0.010	–1.0% [–1.7% to –0.3%]
Observation	0 of 25 (0%)	0 of 53 (0%)	—	—
Outpatient	10 of 4,871 (0.2%)	5 of 2,287 (0.2%)	0.908	0.0% [–0.2% to 0.2%]
Hospital length of stay, days (median [25th, 75th])				
Inpatient	3 [2, 5]	2 [1, 4]	< 0.001	—
Observation	1 [1, 2]	1 [1, 2]	0.545	—
Outpatient	0 [0, 1]	0 [0,1]	< 0.001	—
Discharge disposition, n (%)				
Home	8,554 of 9,034 (94.7%)	3,936 of 4,157 (94.7%)	0.994	0.0% [–0.8% to 0.8%]
Died	4 of 9,034 (0.04%)	2 of 4,157 (0.05%)	0.924	0.0% [–0.08% to 0.08%]
Inpatients readmitted to hospital, n (%)	229 of 4,137 (5.5%)	101 of 1,812 (5.6%)	0.952	0.03% [–1.30% to 1.23%]

P values determined by Mann–Whitney U test for continuous variables and chi-square test for categorical variables. Only data from preimplementation and postimplementation are shown. Median [25th, 75th] indicated for continuous variables; n (%) indicated for categorical variables.

PACU, postanesthesia care unit.

January 2019, the Japanese Society of Anesthesiologists (Kobe, Japan) warned of the dangers of using sugammadex[27] without neuromuscular monitoring, and soon afterward started recommending neuromuscular monitoring when using muscle relaxant antagonists.[28] Guidelines from the United Kingdom,[29] Canada,[30] Australia,[31] and France[32] also advocate neuromuscular monitoring for neuromuscular blocking drug management; none recommend one specific reversal drug for all patients. The reasons for avoidance of monitoring include the belief that it is not necessary, overconfidence in current practice, lack of knowledge/equipment, inability to use equipment, and/or distrust of equipment.[33,34] Contemporary quantitative monitors have been shown relatively easy to use,[35] which contributed to our department-wide adoption.

Acceleromyography yields higher train-of-four ratios compared with electromyography, leading some experts to recommend train-of-four ratios greater than or equal to 1.0 for acceleromyography and train-of-four ratios greater than or equal to 0.9 for electromyography.[1,36,37] Our documentation does not delineate monitor type, so we chose to use the lower cutoff, train-of-four ratio greater than or equal to 0.9, in the context of 24 operating rooms with acceleromyography and 4 with electromyography monitors (with extras of each monitor type also available). Normalizing and preload were not done routinely; therefore, our raw acceleromyography train-of-four ratios between 0.9 to 1.0 may represent incomplete reversal since the Stimpod shows train-of-four ratios greater than 1.0 in approximately one quarter of unparalyzed patients.[38] Numerous studies quantifying residual blockade and assessing postoperative effects have used raw acceleromyography train-of-four ratios greater than or equal to 0.9, supporting our approach.[4,7–9,11,12,39–41] Furthermore, our documentation of acceleromyography measurements before extubation represents a higher standard than PACU measurements, and it saved patients the discomfort of nerve stimulation while awake.[7,39,42]

We analyzed the time from PACU arrival to PACU ready for discharge as an estimate of patient recovery, realizing that

PACU time is affected by many variables. Longer PACU lengths of stay have been reported in the presence of residual blockade.[8,41,43,44] However, our rates of residual paralysis were unknown, so the 7% PACU stay reduction cannot be definitively linked to a reduction in residual paralysis. In 2016, when subjective assessment prevailed, we estimate that one third of our patients likely had residual blockade in the PACU.[45] In June 2019, we evaluated 22 patients arriving to our PACU, and 9% were found to have residual blockade. In December 2020, residual blockade occurred in less than 7% of our patients per operating room train-of-four ratio documentation. In short, we compared populations with inferred, but unknown, rates of residual paralysis.

Recurrent paralysis or regression of the train-of-four ratio after full return of strength was not captured in our study since train-of-four ratios were not measured in the PACU. Neuromuscular blocker duration of action surpassing reversal drug duration of action could lead to recurrent paralysis after neostigmine, but this occurrence is unlikely after a train-of-four ratio greater than or equal to 0.9.[46] Redistribution of neuromuscular blocker molecules from peripheral compartments has been postulated to explain recurrent paralysis after sugammadex.[47] This has generally occurred in the setting of antagonizing deep neuromuscular blockade with low-dose sugammadex. In our study, the average dose of sugammadex given was comparable to manufacturer recommendations, thereby reducing the potential for recurrent paralysis.

A single group before and after analysis lacks a control group, which leaves the conclusions drawn from this type of research vulnerable to confounding factors that threaten the validity of the findings. In our study, many factors exist that impact PACU length of stay, pulmonary complications, and hospital length of stay. We could not control for these factors; therefore, our results must necessarily be viewed with caution. A patient characteristic that could explain improved patient outcomes was that there were 2% fewer inpatient surgeries. Conversely, improved patient outcomes would be less likely with the 10% more ASA Physical Status III to V patients and longer surgical durations present postintervention. Additionally, the anesthesia record data show higher doses of neuromuscular blocker and deeper levels of neuromuscular blockade at the time of reversal. PACU length of stay may have been impacted by intraoperative surgical factors, PACU nursing practices, and PACU-specific quality improvement projects. The change in PACU length of stay may have been due to improved discharge efficiency over time, although this did not occur for patients in our hospital who were managed with a laryngeal mask airway and received no neuromuscular blockade during the same period. Pulmonary complications may be influenced by ventilator management and perioperative pulmonary care practices, but we are unaware of changes in this care occurring between 2016 and 2020. Hospital length of stay was influenced by many factors that were not measured or controlled.

Acceleromyography setup required free movement of the thumb but otherwise closely resembled peripheral nerve stimulator use, a technique familiar to our providers. Frequent error messages and inability to attain measurements historically impeded uniaxial acceleromyography enthusiasm. Introduction of the triaxial monitor, which measured three dimensions of movement, worked more reliably, which facilitated acceleromyography monitor use. We experienced TwitchView array failures that included failed current delivery, failed signal return, poor-quality signal, and inaccurate readings (*e.g.,* train-of-four count 0 with four visible thumb movements). Array failures demotivated providers from using the TwitchView given the cost of each array. This hesitancy was tempered by an agreement with the company to receive two arrays for each that failed (failed array return was required). Our anesthesia technicians were trained on how to place and set up both monitors, and provided reliable, timely, and important assistance. Ongoing equipment upkeep along with responsive device company service contributed immensely to our adoption of quantitative neuromuscular monitoring.

Acceptance of the new standard of train-of-four ratio documentation evolved differently among different anesthesia providers in our department. Residents, in general, were open and receptive to neuromuscular monitor teaching, though monitor utilization was hindered initially by lagging support from some teaching faculty. During supervision, identifying situations when a patient struggled to breathe and subsequently was found by quantitative measurement to have a low train-of-four ratio proved to be a powerful teaching opportunity on multiple occasions. This recognition of patient harm in real time convinced multiple anesthesia providers of the value of monitoring. Our ongoing teaching efforts occurred in departmental meetings, departmental and personal emails, and personal communication. Acceptance occurred differently among anesthesia providers, likely because of the varied preconceived notions about the prevalence of residual paralysis, value of quantitative monitoring, effort required to incorporate quantitative monitoring into practice, and impact of residual paralysis on patients.

We cultivated the mindset of attaining quantitative measurements in part through the implementation of the Ongoing Professional Practice Evaluation, which tied documentation of train-of-four ratios greater than or equal to 0.90 with hospital credentialing. This link established the importance we place on this anesthesiology performance metric. The threshold was set at 70% of cases, but a higher threshold may be warranted. Our practice believes a return-of-strength measurement should be required after every nondepolarizing neuromuscular blocking drug administration.

The improved documentation of train-of-four ratios greater than or equal to 0.9 after incorporation of automated alerts was consistent with known benefits of this technology.[48] We solicited and responded to provider feedback about the alarms to strike a balance between helpful

prompting and alarm fatigue. The optimal delivery of automated prompts will likely differ between institutions.

This work realized the goal of utilizing quantitative monitoring to guide neuromuscular blocker management, which has been advocated in studies, editorials, consensus statements, and practice guidelines. We are continuing our work with anesthesia records that are now electronically searchable to build a dashboard to track documentation of train-of-four ratios greater than or equal to 0.9 in all cases in real time. We are educating providers to document train-of-four ratio in a monitoring field to facilitate electronic searching, as opposed to writing it as a comment in the record. We are working toward automatic data entry into our anesthetic record to streamline the workflow for our providers. Our goal is to make the acquisition of this information seamlessly integrated into the anesthesia care workflow.

Our departmental experience demonstrates that, at the end of surgery in the vast majority of patients, achieving and documenting a train-of-four ratio greater than or equal to 0.9 after administration of a nondepolarizing neuromuscular blocker is not a quixotic goal. This result was achieved in a busy tertiary hospital. However, attaining this endpoint requires more than just placing a quantitative monitor at each anesthetizing location. Ongoing educational effort and follow-up are required. We think our experience provides a useful road map to this end. Anesthesia providers are solely responsible for properly rescuing patients from the states of paralyses they initiate. This should occur for all patients as verified by quantitative measurement and documentation of train-of-four ratios greater than or equal to 0.9.

Acknowledgments

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Competing Interests

The authors declare no competing interests.

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ANESTHESIOLOGY

Amiodarone with or without *N*-Acetylcysteine for the Prevention of Atrial Fibrillation after Thoracic Surgery: A Double-blind, Randomized Trial

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- The American Association for Thoracic Surgery's guidelines for the prevention and management of perioperative atrial fibrillation and flutter provide a class IIa recommendation for administration of amiodarone to prevent postoperative atrial fibrillation in intermediate- and high-risk patients undergoing lung resection and esophagectomy
- However, approximately 15% of patients receiving amiodarone still develop postoperative atrial fibrillation
- *N*-Acetylcysteine has anti-inflammatory properties, but its efficacy in reducing atrial fibrillation in noncardiac thoracic surgical patients has not been well studied

What This Article Tells Us That Is New

- This double-blinded randomized trial of noncardiac thoracic surgery patients was done to test the hypothesis that the addition of *N*-acetylcysteine to concurrent amiodarone administration would reduce the incidence of postoperative atrial fibrillation when compared with placebo being concurrently administered with amiodarone
- The study was halted midway for futility, as there was no difference in postoperative atrial fibrillation in the patients who received *N*-acetylcysteine plus amiodarone *versus* the patients who received placebo plus amiodarone

ABSTRACT

Background: Postoperative atrial fibrillation may identify patients at risk of subsequent atrial fibrillation, with its greater risk of stroke. This study hypothesized that *N*-acetylcysteine mitigates inflammation and oxidative stress to reduce the incidence of postoperative atrial fibrillation.

Methods: In this double-blind, placebo-controlled trial, patients at high risk of postoperative atrial fibrillation scheduled to undergo major thoracic surgery were randomized to *N*-acetylcysteine plus amiodarone or placebo plus amiodarone. On arrival to the postanesthesia care unit, *N*-acetylcysteine or placebo intravenous bolus (50 mg/kg) and then continuous infusion (100 mg/kg over the course of 48 h) was administered plus intravenous amiodarone (bolus of 150 mg and then continuous infusion of 2 g over the course of 48 h). The primary outcome was sustained atrial fibrillation longer than 30 s by telemetry (first 72 h) or symptoms requiring intervention and confirmed by electrocardiography within 7 days of surgery. Systemic markers of inflammation (interleukin-6, interleukin-8, tumor necrosis factor α, C-reactive protein) and oxidative stress (F_2-isoprostane prostaglandin $F_{2\alpha}$; isofuran) were assessed immediately after surgery and on postoperative day 2. Patients were telephoned monthly to assess the occurrence of atrial fibrillation in the first year.

Results: Among 154 patients included, postoperative atrial fibrillation occurred in 15 of 78 who received *N*-acetylcysteine (19%) and 13 of 76 who received placebo (17%; odds ratio, 1.24; 95.1% CI, 0.53 to 2.88; $P = 0.615$). The trial was stopped at the interim analysis because of futility. Of the 28 patients with postoperative atrial fibrillation, 3 (11%) were discharged in atrial fibrillation. Regardless of treatment at 1 yr, 7 of 28 patients with postoperative atrial fibrillation (25%) had recurrent episodes of atrial fibrillation. Inflammatory and oxidative stress markers were similar between groups.

Conclusions: Dual therapy comprising *N*-acetylcysteine plus amiodarone did not reduce the incidence of postoperative atrial fibrillation or markers of inflammation and oxidative stress early after major thoracic surgery, compared with amiodarone alone. Recurrent atrial fibrillation episodes are common among patients with postoperative atrial fibrillation within 1 yr of major thoracic surgery.

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Transient atrial fibrillation after noncardiac thoracic or general surgery has long been thought to be a benign and self-limited disorder; however, new evidence suggests it may be a sentinel event that can be used to identify patients who are at risk of developing subsequent atrial fibrillation and who have a greater risk of short- and long-term stroke.[1–4] Postoperative atrial fibrillation may have hemodynamic consequences and result in treatment-related adverse events, such as bleeding, drug toxicity, and extended hospital stay of 2 to 4 days, with greater average cost of care of more than 30%.[1] As is the case for atrial fibrillation not related to surgery, age

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greater than 60 yr is strongly associated with postoperative atrial fibrillation. Other proven risk factors in observational studies of atrial fibrillation after noncardiac thoracic surgery include history of atrial fibrillation, extent of surgery, and preoperative subclinical higher brain natriuretic peptide level.[5,6] Proposed pathophysiologic changes underlying heightened risk of atrial fibrillation after noncardiac thoracic surgery include oxidative stress, inflammation, autonomic imbalance, and damage to autonomic fibers.[1] The onset of arrhythmia peaks 2 to 3 days after surgery, with approximately 85% of these episodes reverting to sinus rhythm by the use of rate or rhythm control strategies during hospitalization.[1] Amiodarone has received a class IIa recommendation from the American Association for Thoracic Surgery (Beverly, Massachusetts) Taskforce for the prevention of postoperative atrial fibrillation in intermediate- and high-risk patients undergoing lung resection and esophagectomy.[1] More than 15% of patients at high risk will experience atrial fibrillation after thoracic surgery when amiodarone is used for prevention, and amiodarone may be associated with transient bradycardia, hypotension, and, rarely, pulmonary toxicity.[1,7] An effective treatment that specifically targets inflammation and oxidative distress to reduce the incidence of postoperative atrial fibrillation has been elusive.

N-Acetylcysteine is an antioxidant anti-inflammatory agent with a demonstrated effect on proinflammatory cytokines, oxygen-free radicals, and ischemia reperfusion injury. In a limited number of studies, intravenous *N*-acetylcysteine has been shown to reduce the incidence of postoperative atrial fibrillation related to cardiac surgery,[8,9] but it has not been tested in patients undergoing noncardiac thoracic surgery. Oxidative stress in patients undergoing thoracic surgery has been reported, with one-lung ventilation routinely used to facilitate operative conditions.[10] Furthermore, *in vitro* studies have shown that *N*-acetylcysteine reduced inflammation and oxidative stress in response to injury in human airway smooth muscle cells.[11] In patients undergoing major thoracic surgery who were identified to have a greater risk of postoperative atrial fibrillation by use of a novel prediction tool,[5,6] we tested the hypothesis that the addition of *N*-acetylcysteine to amiodarone would reduce the incidence of postoperative atrial fibrillation, compared with amiodarone alone.

Materials and Methods

Study Design and Participants

The current study was an investigator-initiated, randomized, double-blind, placebo-controlled trial comparing intravenous *N*-acetylcysteine plus amiodarone with *N*-acetylcysteine–matched placebo plus amiodarone conducted at two centers (Memorial Sloan Kettering Cancer Center [New York, New York] and Washington University Medical Center [St. Louis, Missouri]; ClinicalTrials.gov identifier, NCT02750319; https://clinicaltrials.gov/ct2/show/NCT02750319; principal investigator, David Amar; date of registration, April 25, 2016; full protocol available on request). Eligible patients were aged 18 yr or older, were scheduled for elective major thoracic surgery (anatomic pulmonary resection [segmentectomy, lobectomy, bilobectomy, or pneumonectomy] or esophagectomy), were in sinus rhythm preoperatively, and had one of the following four risk criteria: (1) female sex and preoperative brain natriuretic peptide of 25 pg/ml or greater; (2) male sex, age less than 75 yr, and preoperative brain natriuretic peptide 25 pg/ml or greater; (3) male sex and age 75 yr or older; and (4) history of atrial fibrillation. As patients undergoing Ivor Lewis esophagectomy consisting of abdominal and right thoracic cavity incisions often experience postoperative atrial fibrillation,[1] these patients were included as well. The risk criteria were derived from our previously published work, which showed a higher incidence of atrial fibrillation in men, those age 75 yr or older, and those with a history of atrial fibrillation; brain natriuretic peptide 25 pg/ml or greater (median for cohort) helped distinguish risk among women and men age less than 75 yr.[5,6] The brain natriuretic peptide measurements were performed using an Alere triage meter (Alere North America, USA) at both institutions. The exclusion criteria were hemodynamic instability, second-degree atrioventricular block, hypersensitivity to amiodarone or *N*-acetylcysteine, current use of class Ic or III antiarrhythmic drugs, hepatic insufficiency (more than 2.0 times the

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upper limit of normal of transaminase levels), renal insufficiency (creatinine 2.0 mg/dl or greater), or pregnancy. To avoid withdrawal symptoms, preoperative β-blockers and calcium channel blockers were continued postoperatively. Potential candidates were screened for eligibility by dedicated research staff who introduced the study to patients, written informed consent was obtained by the attending surgeon from each patient, and the study was approved by the institutional review boards of Memorial Sloan Kettering Cancer Center and Washington University Medical Center.

Randomization

Patients underwent routine preoperative evaluation, including brain natriuretic peptide measurement, and were randomized 1:1 to a study group before the day of surgery. The Memorial Sloan Kettering Cancer Center statisticians responsible for randomization (independent of the study statistician) set up the parameters for the allocation sequence, which occurred in real time once patients were registered as eligible and consented to the trial. Randomization was accomplished by computer algorithm using the method of random permuted blocks (of random sizes of 2, 4, or 6), stratified by procedure type (lung resection or esophagectomy). The randomization schedule was concealed from Memorial Sloan Kettering Cancer Center investigators by use of a centralized randomization module that generated allocations in real time only to Memorial Sloan Kettering Cancer Center hospital pharmacists. The randomization schedule was concealed from Washington University investigators by use of a centralized randomization module housed at Memorial Sloan Kettering Cancer Center, which generated allocations in real time only to Memorial Sloan Kettering Cancer Center statisticians responsible for randomization, a team independent of the study statistician. The sequence of assignments for Memorial Sloan Kettering Cancer Center patients was administered in real time to Memorial Sloan Kettering Cancer Center hospital pharmacists, who dispensed the study drugs. The sequence of assignments for Washington University patients was administered in real time to Memorial Sloan Kettering Cancer Center randomization statisticians, who then securely emailed the assignments only to the Washington University pharmacist who dispensed the study drugs.

Interventions

Anesthesia and surgical techniques followed standard institutional care. Anesthesia was induced with midazolam, propofol, and fentanyl and maintained with volatile anesthetics and a nondepolarizing muscle relaxant. Epidural analgesia was used during and after surgery only for patients who underwent open surgical procedures. Surgical procedures for lung resection included both open thoracotomy and minimally invasive video-assisted thoracoscopic or robotic-assisted approaches and Ivor Lewis esophagectomy consisting of abdominal and right thoracic cavity incisions using either minimally invasive laparoscopy and thoracoscopy or open incisions. No robotic esophagectomies were included. Patients who underwent ineligible surgical procedures (*e.g.*, lung wedge resection or biopsy only) that were protocol-prespecified as ineligible were not treated in the study, taken off the study, and removed from all analyses.

The study drug and placebo were prepared at both sites by the institutional pharmacy, and all investigators, clinical care team members, and patients were blinded to treatment allocation. All patients received amiodarone, as per routine practice, as follows—loading dose: 150 mg of amiodarone intravenously in the postanesthesia care unit over the course of 1 h and then 2 g over the course of 48 h. The patients were randomized to receive either *N*-acetylcysteine loading dose 50 mg/kg intravenously in the postanesthesia care unit over the course of 1 h and then by continuous infusion 100 mg/kg over the course of 48 h or matching saline placebo. The selected dose of *N*-acetylcysteine has been described.[8,9] We chose not to administer *N*-acetylcysteine before surgery to avoid the exclusion of treated patients from the primary analysis who later were found to have undergone ineligible procedures. The patients were monitored with continuous telemetry for a minimum of 72 h or longer if needed. Rhythm strips from the telemetry system and the QT interval in response to drug therapy were monitored in accordance with the standard of care.

All patients who developed acute postoperative atrial fibrillation and did not respond to first-line drugs for rate control, such as diltiazem or metoprolol, were managed by a consulting cardiologist. The CHA_2D_2S-VASc (congestive heart failure, hypertension, age 75 yr or older, diabetes mellitus, stroke or transient ischemic attack, vascular disease, age 65 to 74 yr, sex category [female]) score for each patient was recorded. For patients with arrhythmia less than 48 h in duration, anticoagulant therapy was considered after weighing the risk of postoperative bleeding, but ultimately the decision was left to the treating physician. Anticoagulation therapy was considered for patients with a CHA_2D_2S-VASc score of 1 and was recommended for those with a score of 2 or higher. Patients with postoperative atrial fibrillation more than 48 h in duration received anticoagulant therapy unless the risk of postoperative bleeding was judged to be too high.[1]

Outcomes

The primary outcome was new onset of either sustained postoperative atrial fibrillation (greater than 30 s) detected by telemetry (first 72 h) or symptomatic postoperative atrial fibrillation requiring intervention and documented by 12-lead electrocardiography within 7 days of surgery. The primary outcome was verified by a clinical cardiologist and investigators blinded to the treatment assignment. Secondary outcomes were differences between the groups in perioperative systemic markers of inflammation and

oxidative stress and incidence of atrial fibrillation up to 1 yr after discharge regardless of treatment assignment. At the time of hospital discharge, patients who developed postoperative atrial fibrillation in-hospital were equipped with mobile electrocardiography monitors (AliveCor, USA) that adhere to a smart phone or tablet and were instructed to transmit weekly electrocardiogram recordings for up to 1 yr from surgery. Continuous heartbeat data (rhythm strip) were recorded and transmitted wirelessly in real time. All patients, regardless of whether they developed postoperative atrial fibrillation, were contacted by phone at 7, 14, and 30 days and then monthly for the first year after surgery. We reviewed the subsequent follow-up physician visits and electronic medical records of all study patients for any evidence of hospitalization or treatment for new-onset atrial fibrillation. Length of hospital stay and 30- and 90-day mortality were recorded from a regularly maintained hospital database. Additional secondary outcomes were cardiovascular complications (including myocardial infarction, stroke, thromboembolic events, and major bleeding requiring reoperation) and pulmonary complications (including atelectasis, pneumonia, pneumonitis, and acute respiratory failure). Treatment-related adverse events, such as allergic reaction to *N*-acetylcysteine or transient hypotension or bradycardia with amiodarone, were recorded.

Biospecimen Analysis

To assess markers of inflammation and oxidative stress, plasma and urine samples, respectively, were collected at the end of surgery and again on the morning of postoperative day 2 from all patients. Interleukin-6, interleukin-8, and tumor necrosis factor α were measured by enzyme-linked immunosorbent assay (Ella Simple Plex, ProteinSimple, USA). C-reactive protein was measured by means of an immunoturbidometric method using the Abbott Architect analyzer (USA). Urine samples were collected to assess systemic markers of oxidative stress (urinary F_2-isoprostane prostaglandin $F_{2\alpha}$ and isofuran). Measurements of these markers were assessed by the use of specific gas chromatography–mass spectrometry in the Eicosanoid Core Laboratory (Vanderbilt University Medical Center, by G.L.M.), as previously described.[12] Plasma and urine samples were processed similarly at both study sites and stored at –80°C until analysis. Planned analyses of samples from both sites, in duplicate, were to be performed for plasma inflammatory markers at Memorial Sloan Kettering Cancer Center and for urine oxidative markers at Vanderbilt University Medical Center.

Statistical Analysis

Sample-size calculations were based on an anticipated proportion of patients with postoperative atrial fibrillation of 16% in the placebo group and 5% in the *N*-acetylcysteine group (absolute decrease of 11% and relative difference of 69%). The hypothesized rate of 5% postoperative atrial fibrillation is the rate among low-risk patients in the population based on historical data.[5] Furthermore, the hypothesized relative difference of 69% (11 of 16) was based on the approximately 65% response rate reported in published studies using *N*-acetylcysteine.[8,9] A sample size of 244 evaluable patients (122 patients in each group) would therefore achieve 80% power at a two-sided 5% α level. One formal interim analysis was planned halfway through enrollment (122 evaluable patients), using a Lan–DeMets spending function approach with O'Brien–Fleming stopping boundaries for both efficacy and futility. At the interim analysis, if $P \leq 0.002$, enrollment would be discontinued, and we would conclude that amiodarone plus *N*-acetylcysteine significantly decreased the rate of postoperative atrial fibrillation, compared with amiodarone alone (efficacy). If $P \geq 0.719$, enrollment would be stopped for futility. If $0.002 < P < 0.719$, the trial would continue to full enrollment, and we would conclude that *N*-acetylcysteine significantly reduced postoperative atrial fibrillation if $P < 0.049$. The planned interim analysis was conducted by the study statistician without knowledge of the treatment group labels.

On February 5, 2019, an interim analysis was conducted on 122 evaluable patients with primary endpoints, as detailed in the previous paragraph. Comparison of the two groups in the overall cohort indicated that the futility boundary was crossed. However, the chair of the Data Safety Monitoring Board, who reviewed the interim analysis results, recommended that the study continue accruing patients until all members of this committee could review the updated data at the annual meeting. During the Data Safety Monitoring Board meeting on June 20, 2019, the committee reviewed the updated results and recommended that the study be terminated due to futility. The study thus stopped enrolling patients at that point, with 154 evaluable patients. All data reported herein are based on the patients accrued up to that point.

The primary analysis was conducted using the intention-to-treat principle. Evaluable patients were randomized and had the primary endpoint available. Ideally, randomization would have occurred intraoperatively after confirming that the patient's final surgical procedure met the eligibility criteria of either anatomical lung resection or esophagectomy. However, intraoperative randomization was not feasible, given the need to ensure the same randomization time point and accommodate logistic considerations at both study sites. The decision process to withdraw randomized patients intraoperatively because of violation of eligibility criteria was prespecified in the protocol and followed objective protocol-based criteria before initiation of treatment and without knowledge of the assigned group. Exclusion of these patients from the analysis was deemed appropriate and consistent with statistical principles of clinical trials.[13,14] Other postrandomization exclusions included

cancelled surgery and patient withdrawal before surgery, both of which would not provide study endpoints.

Continuous data were summarized as median (25th to 75th percentile) and categorical data as frequency (percentage). The proportions of patients with postoperative atrial fibrillation were summarized and compared between the two groups using the Mantel–Haenszel test of homogeneity of odds ratio across the stratification factor of procedure type (lung resection or esophagectomy). Length of hospital stay was compared between groups using zero-truncated Poisson regression models. Incidences of cardiovascular and pulmonary adverse events and 30- and 90-day mortality were compared between the groups using the chi-square test. With an overall study-wise type I error (α) set to 0.05 (two-sided), the α for the final analysis of the primary endpoint was 0.049, and thus, 95.1% CI values were used in reporting the odds ratios from the primary outcome analysis. For all other analyses, no adjustments were made, and an α level of 0.05 was used, along with corresponding 95% CI when reporting contrasts. All analyses were two-sided and conducted using Stata 15.1 (StataCorp, USA).

Results

Patient Characteristics

Between September 16, 2016, and July 18, 2018, 308 patients were enrolled, and 188 were randomized (95 in the *N*-acetylcysteine group, 93 in the placebo group; fig. 1). In the *N*-acetylcysteine group, 17 patients were excluded; in the placebo group, 17 were excluded (reasons summarized in fig. 1). In total, the population for analysis included 154 evaluable patients (78 in the *N*-acetylcysteine group, 76 in the placebo group). The distribution of patient clinical and surgical characteristics is summarized in table 1. All patients in the analysis set are from Memorial Sloan Kettering Cancer Center. The Washington University Medical Center site randomized one patient, who was later found to be ineligible intraoperatively and was removed from the study before any treatment. All 154 evaluable patients included in the analysis had complete primary and secondary endpoints, except for inflammatory and oxidative stress markers on postoperative day 2 (missing in 16). Demographic and clinical data were complete for all evaluable patients, except for predicted forced expiratory volume in 1 s (FEV_1; missing in 39) and predicted diffusing capacity for carbon monoxide (%; missing in 42; table 1). None of these baseline factors was necessary for stratification or hypothesis testing.

Primary Outcome

Postoperative atrial fibrillation occurred in 28 of the 154 patients (18%), at a median of 2 days after surgery, with a median (25th to 75th percentile) duration of 12 (2 to 49) h. One patient had mixed atrial fibrillation and atrial flutter. The incidence of postoperative atrial fibrillation was not statistically significantly different between the two groups after stratification by procedure type (19% [15 of 78] in the *N*-acetylcysteine group *vs.* 17% [13 of 76] in the placebo group; odds ratio, 1.24; 95.1% CI, 0.53 to 2.88; $P = 0.615$; table 2). The first interim analysis was performed as planned after 122 patients (50% of the maximum sample size) had completed their 30-day follow-up in February 2018. The Data and Safety Monitoring Board met annually, and at their recommendation, the trial was stopped for futility after the interim analysis.

The median (25th to 75th percentile) day of onset of postoperative atrial fibrillation was not statistically significantly different between the *N*-acetylcysteine group (2 [1 to 3] days) and the placebo group (2 [2 to 4] days; $P = 0.106$). One patient developed postoperative atrial fibrillation after discharge on postoperative day 4. In 14 of the 28 patients with postoperative atrial fibrillation (50%; 8 in the *N*-acetylcysteine group and 6 in the placebo group), postoperative atrial fibrillation was symptomatic. None of the patients with postoperative atrial fibrillation required emergency electrical cardioversion. The rate of atrial fibrillation was not significantly different between those who received β-blockers and those who did not (22% *vs.* 16%; $P = 0.504$) or between those who received calcium blockers and those who did not (20% *vs.* 18%; $P = 0.804$).

Secondary Outcomes

Of the 28 patients with postoperative atrial fibrillation, 4 (14%) received oral anticoagulants after surgery and remained on oral anticoagulant therapy at the time of discharge. Of these, 3 remained in atrial fibrillation, and 1 had a history of paroxysmal atrial fibrillation and was discharged in sinus rhythm. At 1 yr of follow-up, 7 of the 28 patients with postoperative atrial fibrillation (25%) had recurrent episodes of atrial fibrillation, and 1 patient had persistent atrial fibrillation. None of these patients developed a stroke, as determined from regular phone follow-ups and review of the medical record during the first year after surgery. Of the 28 patients with postoperative atrial fibrillation, 16 (57%) were compliant with ambulatory monitoring guidelines. Among the 7 patients with recurrent atrial fibrillation, diagnosis was made by ambulatory monitoring in 3 and by phone follow-up and electronic medical record surveillance in 4. Within 1 yr of surgery, no patient without postoperative atrial fibrillation reported being treated for symptomatic atrial fibrillation episodes. Length of hospital stay and mortality were similar between the groups, as were other secondary outcomes (table 2).

Treatment-related Adverse Events

Adverse outcomes were infrequent and were not attributed to the experimental therapy. One patient developed signs and symptoms of a severe allergic reaction near the conclusion of study medication infusion, which the surgical

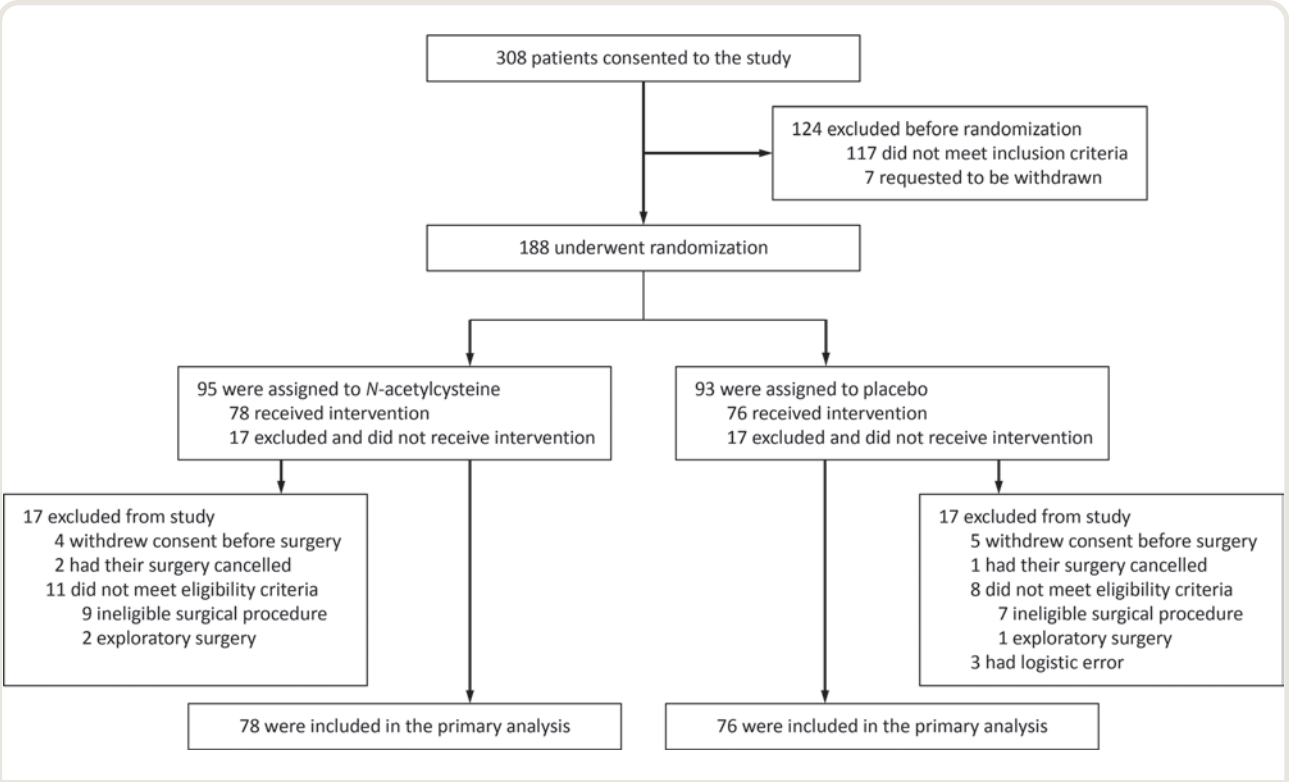

Fig. 1. Consolidated Standards of Reporting Trials diagram of the study population.

team attributed to oxycodone ingestion. The infusion of amiodarone was associated with hypotension and was transiently stopped or terminated in 31 of the 154 patients (20%; 16 in the *N*-acetylcysteine group and 15 in the placebo group). The amiodarone infusion was also transiently stopped because of bradycardia (heart rate less than 50 beats/min) in 3 of the 154 patients (2%; all in the placebo group).

Inflammatory and Oxidative Stress Markers

The levels of C-reactive protein, tumor necrosis factor α, interleukin-6, and interleukin-8 were similar between the groups immediately after surgery and on postoperative day 2 (table 3). Moreover, urinary isoprostane and isofuran levels were not statistically different immediately after surgery or on postoperative day 2 (table 3).

Discussion

To our knowledge, this is the first trial to investigate *N*-acetylcysteine plus amiodarone to prevent postoperative atrial fibrillation in patients undergoing anatomical lung or esophageal resection determined to be at high risk of postoperative atrial fibrillation by use of novel clinical and biomarker criteria. With amiodarone prophylaxis, the overall incidence of postoperative atrial fibrillation was 18% in our study and near the anticipated rate of 16% used for our sample size calculation for the placebo group. The addition of *N*-acetylcysteine to amiodarone did not reduce the incidence of postoperative atrial fibrillation; we therefore terminated the study early for futility after the interim analysis. The compelling rationale for testing *N*-acetylcysteine in patients undergoing noncardiac thoracic surgery is that *N*-acetylcysteine had been demonstrated to robustly reduce postoperative atrial fibrillation related to cardiac surgery when used alone or in addition to other drugs.[8,9] Because cardiac and thoracic surgery share some similar intraoperative surgical stressors that may contribute to postoperative atrial fibrillation, we hypothesized that the addition of the antioxidant *N*-acetylcysteine to the multichannel antiarrhythmic drug amiodarone would provide a synergistic and more effective prevention regimen against postoperative atrial fibrillation than amiodarone alone. We acknowledge that *N*-acetylcysteine administration before surgery could have mitigated inflammatory and oxidative stressors and affected our results; however, we chose to wait to include patients until establishing that their cancer operation could be performed and that they met the study eligibility criteria. Earlier meta-analyses reporting the effects of *N*-acetylcysteine on cardiac surgery–related postoperative atrial fibrillation showed that *N*-acetylcysteine reduced the incidence of atrial fibrillation after heart surgery and all-cause mortality, compared with controls.[15] More recently,

Table 1. Patient Characteristics by Treatment Group

Characteristic	*N*-Acetylcysteine (n = 78; 51%)	Placebo (n = 76; 49%)	Absolute Standardized Mean Difference
Age, yr	71 (67–76)	70 (67–76)	0.061
Male sex	39 (50)	43 (57)	0.131
Body mass index	27.7 (23.8–32.5)	27.3 (24.2–30.1)	0.015
ASA physical status			0.283
II	2 (2.6)	7 (9.2)	
III	76 (97)	69 (91)	
Coronary artery disease	15 (19)	19 (25)	0.138
Hypertension	60 (77)	53 (70)	0.162
Chronic obstructive pulmonary disease	18 (23)	17 (22)	0.017
Diabetes	14 (18)	16 (21)	0.078
Smoking history	64 (82)	62 (82)	0.012
History of atrial fibrillation	5 (6.4)	5 (6.6)	0.007
Preoperative chemotherapy	22 (28)	25 (33)	0.101
Preoperative radiotherapy	12 (15)	16 (21)	0.146
Medications			
β-Blocker	28 (36)	22 (29)	0.148
Angiotensin-converting enzyme/angiotensin II receptor blocker	18 (23)	20 (26)	0.075
Calcium channel blocker	20 (26)	15 (20)	0.140
Statin	41 (53)	44 (58)	0.107
Brain natriuretic peptide, pg/ml	55 (36–88)	52 (33–92)	0.060
FEV_1 predicted (n = 115), %	0.9 (0.8–1.0)	0.9 (0.8–1.0)	0.027
Missing	17	22	
Lung diffusion capacity for carbon monoxide predicted (n = 112), %	0.9 (0.7–1.0)	0.8 (0.7–1.0)	0.056
Missing	19	23	
CHA_2D_2S-VASc score			0.109
< 2	9 (12)	10 (13)	
2 to 3	48 (62)	49 (64)	
≥ 4	21 (27)	17 (22)	
Procedure			0.106
Lung resection	62 (79)	57 (75)	
Lobectomy	50	50	
Pneumonectomy	0	1	
Segmentectomy	12	6	
Esophagectomy	16 (21)	19 (25)	
Minimally invasive surgery	68 (87)	60 (79)	0.219
Lung resection (n = 119)	62	57	
Open	5 (8)	9 (16)	
Video-assisted thoracic surgery	28 (45)	18 (31)	
Robotic-assisted thoracic surgery	29 (47)	30 (53)	
Esophagectomy (n = 35)	16	19	
Open	5 (31)	7 (37)	
Minimally invasive	11 (69)	12 (63)	

The data are presented as no. (%) or median (25th to 75th percentile). All patients in the analysis set are from Memorial Sloan Kettering Cancer Center. The Washington University Medical Center site randomized one patient, who was later found to be ineligible intraoperatively and was removed from the study before any treatment.

ASA, American Society of Anesthesiologists; CHA_2D_2S-VASc, congestive heart failure, hypertension, age 75 yr or more, diabetes mellitus, stroke or transient ischemic attack, vascular disease, age 65 to 74 yr, sex category (female); FEV_1, forced expired volume in 1 s.

a meta-analysis of randomized controlled trials, published shortly after the completion of this randomized controlled study, showed that the addition of *N*-acetylcysteine during cardiac surgery did not meaningfully reduce clinically important outcomes such as arrhythmia, length of hospital stay, or mortality.[16] Larger studies have found conflicting results regarding whether perioperative supplementation with oral n-3 polyunsaturated fatty acids and/or vitamin C or E in patients undergoing cardiac surgery is associated with a meaningful reduction in the incidence of postoperative atrial fibrillation.[17,18]

Among the patients with postoperative atrial fibrillation in our study, 25% had recurrence of atrial fibrillation within 1 yr of surgery. Importantly, no patients with postoperative atrial fibrillation developed a stroke. However, our findings likely underreported the true rate of subsequent atrial fibrillation, as more than 40% of our patients were not compliant with the ambulatory monitoring protocol. In a similar population to ours, Higuchi *et al.*[19] used ambulatory event recorders and demonstrated a rate of atrial fibrillation recurrence of 30% in patients who developed postoperative atrial fibrillation within 1 yr of cancer surgery (65% of these patients

Table 2. Outcomes by Treatment Group

Variable	*N*-Acetylcysteine (n = 78; 51%)	Placebo (n = 76; 49%)	Odds Ratio (95% CI)*	*P*†
Primary endpoint: postoperative atrial fibrillation within 7 days				
All patients	15/78 (19)	13/76 (17)	1.24 (0.53–2.88)‡	0.615
Lung resection (n = 119)	8/62 (13)	8/57 (14)	0.91 (0.272–3.03)	
Esophagectomy (n = 35)	7/16 (44)	5/19 (26)	2.18 (0.42–11.6)	
Secondary endpoints				
Symptomatic postoperative atrial fibrillation	8 (10)	6 (7.9)	1.33 (0.44–4.0)	
Recurrent atrial fibrillation within 1 yr	3/15 (20)	4/13 (31)	—	
Length of hospital stay, days				
All patients	5 (3–8)	6 (4–9)	0.97 (0.78–1.21)	
Lung resection	4 (3–6)	5 (2–7)	—	
Esophagectomy	8 (7–15)	9 (7–10)	—	
Pulmonary complications§	5 (6.4)	6 (7.9)	0.80 (0.233–2.74)	
Reoperation for bleeding	0 (0)	2 (2.6)	—	
Transient ischemic attack	1 (1.3)	0 (0)	—	
Venous thromboembolism	1 (1.3)	3 (3.9)	—	
Overall mortality				
30-day	0 (0)	0 (0)	—	
90-day	1 (1.3)	1 (1.3)	—	

Summary values are presented as no. (%) or median (25th to 75th percentile).

*Estimates for *N*-acetylcysteine *versus* placebo contrasts were extracted from logistic regression models for dichotomous outcomes (odds ratios, for outcomes with at least 10 events) and linear regression models for log-transformed length of stay outcome (coefficient, percentage increase in length of stay). As an adjustment for the interim analysis, 95.1% CI values are presented for the primary endpoint; all others are 95% CI. †*P* value is presented for the primary outcome from the Mantel–Haenszel test of homogeneity of odds ratio across the stratification factor of procedure type. A descriptive summary of exploratory secondary outcomes is shown. Given the potential for type I error due to multiple comparisons and the low event rate, no formal hypothesis testing was conducted for secondary outcomes. ‡Mantel–Haenszel estimate of the odds ratio stratified by procedure type. §Atelectasis, pneumonia, pneumonitis, and respiratory failure.

Table 3. Inflammatory and Oxidative Stress Markers by Treatment Groups

Marker	*N*-Acetylcysteine (n = 78; 51%)	Placebo (n = 76; 49%)	Coefficient (95% CI)*
C-reactive protein, pg/ml			
Immediately after surgery	0.3 (0.1 to 0.6)	0.3 (0.1 to 0.7)	
Postoperative day 2 (n = 139)	14.7 (8.8 to 21.6)	12.3 (9.0 to 18.0)	1.2 (–1.3 to 3.7)
Interleukin-6, pg/ml			
Immediately after surgery	98.0 (57.0 to 160.0)	78.0 (43.5 to 166.0)	
Postoperative day 2 (n = 139)	73.5 (45.0 to 142.1)	83.0 (36.0 to 135.0)	5.9 (–29.7 to 41.4)
Interleukin-8, pg/ml			
Immediately after surgery	21.9 (14.5 to 32.8)	19.8 (14.0 to 36.0)	
Postoperative day 2 (n = 139)	25.8 (16.0 to 42.4)	28.5 (19.6 to 39.8)	–3.5 (–10.6 to 3.6)
Tumor necrosis factor α, pg/ml			
Immediately after surgery	7.0 (5.3 to 8.0)	6.7 (5.0 to 8.0)	
Postoperative day 2 (n = 139)	9.0 (7.0 to 12.0)	9.0 (7.0 to 10.0)	1.4 (–0.7 to 3.4)
F_2-isoprostane prostaglandin $F_{2\alpha}$, ng/mg creatinine†			
Immediately after surgery	1.4 (0.8 to 2.2)	1.4 (1.0 to 1.9)	
Postoperative day 2 (n = 138)	0.9 (0.7 to 1.4)	0.9 (0.6 to 1.3)	0.0 (–0.2 to 0.2)
Isofuran, ng/mg creatinine†			
Immediately after surgery (n = 153)	1.9 (1.4 to 3.0)	2.0 (1.5 to 2.9)	
Postoperative day 2 (n = 138)	1.6 (1.2 to 2.1)	1.6 (1.1 to 2.2)	0.0 (–0.3 to 0.3)

The values are presented as medians (25th to 75th percentile).

*Coefficients and corresponding 95% CI values are extracted from linear regression models with placebo as the reference group and adjustment for immediately after surgery marker values. †According to standard laboratory methods, these values have been adjusted for urine creatinine concentration and reported as ng/mg creatinine before statistical analyses.

had undergone thoracic surgery). In a retrospective study of a defined population of patients who underwent noncardiac surgery, development of subsequent atrial fibrillation was more common among patients who had postoperative atrial fibrillation than among patients who did not have postoperative atrial fibrillation.[4] Several observational studies using large databases have shown a clear link between postoperative atrial fibrillation after noncardiac surgery and subsequent

development of stroke and thromboembolic events within 1 to 5 yr after the index surgery.[2,20,21] Two of these studies also found an association between postoperative atrial fibrillation and higher risk of death and myocardial infarction. Using the Danish nationwide registry, Butt *et al.*[3] matched patients who developed postoperative atrial fibrillation with patients who had nonvalvular atrial fibrillation not related to surgery and found that the long-term risk of thromboembolism was similar between these two populations. The implications of our results and those of the above studies on further management of postoperative atrial fibrillation, such as the need for anticoagulation, require investigation in future randomized trials.

Laboratory and human tissue studies have shown an association between higher levels of inflammation and monoamine oxidase and NADPH oxidase activity and the development of postoperative atrial fibrillation after cardiac surgery.[22,23] In a large study of patients who underwent cardiac surgery, urinary isoprostane and isofuran levels were associated with postoperative atrial fibrillation in the perioperative period, whereas plasma concentrations were not found to be associated with postoperative atrial fibrillation.[24] That the use of *N*-acetylcysteine was not associated with lower markers of inflammation and oxidative stress may help to explain the lack of a reduction in postoperative atrial fibrillation in this study.

Limitations

Our study had several limitations. First, we did not include a third group of patients who received only placebo—that is, no amiodarone—as our standard of care was to include postoperative atrial fibrillation prevention for at-risk patients.[1,5–7] Second, the duration of *N*-acetylcysteine infusion used in our trial could have been longer; however, we sought to administer the drug during the peak time that patients are most likely to develop postoperative atrial fibrillation, which is up to 48 h postoperatively. Third, ambulatory monitors were provided only to patients who developed postoperative atrial fibrillation; therefore, the rate of atrial fibrillation among patients without postoperative atrial fibrillation is not known. We did, however, contact all patients by phone at 7, 14, and 30 days and monthly for the first year after the surgery and reviewed the electronic medical records of all study patients for any evidence of hospitalization or treatment for new-onset atrial fibrillation, and no subsequent atrial fibrillation in patients without postoperative atrial fibrillation was reported. Last, we were not able to accrue enough patients from our second site (Washington University Medical Center), mostly because of administrative delays.

Conclusions

N-Acetylcysteine was not associated with a lower incidence of atrial fibrillation within 7 days after anatomical lung resection or esophagectomy. Recurrent episodes of atrial fibrillation confirmed by either mobile electrocardiography monitoring or symptomatically within 1 yr of noncardiac thoracic surgery were common among patients who developed postoperative atrial fibrillation.

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Competing Interests

Dr. Park has served as a proctor for Intuitive Surgical (Sunnyvale, California) and consultant for COTA (New York, New York). Dr. Isbell is a consultant for Genentech (San Francisco, California), has an equity interest in LumaCyte LLC (Charlottesville, Virginia), and has a financial relationship with Guardant Health (Redwood City, California). Dr. Molena is a consultant for Johnson & Johnson (New Brunswick, New Jersey), Urogen (New York, New York), Intuitive Surgical, and Boston Scientific (Marlborough, Massachusetts). Dr. Fischer is a consultant for Edward Lifesciences (Irvine, California). Dr. Rusch reports grant support (institutional) from Genelux (San Diego, California) and Genentech, travel support from Intuitive Surgical, and travel support and payments from the National Institutes of Health Coordinating Center for Clinical Trials. Dr. Jones serves as a consultant for AstraZeneca (Cambridge, United Kingdom) and is on a clinical trial steering committee for Merck (Kenilworth, New Jersey). The other authors declare no competing interests.

Reproducible Science

Full protocol available at: amard@mskcc.org. Raw data available at: amard@mskcc.org.

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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Ethereal Breath of the Gods: Always Circulating Aither

Humans breathe air. In contrast, the gods of ancient Greek mythology inspired *aither*, *aether*, or *ether*. Rather than the transparent gas inhaled by mortals, the deities breathed the blue gas found on mountain tops and in the heavens—all that lofty atmosphere above ground but beneath the solid dome of the Sky (*Ouranos*, Latinized as Uranus). Unlike earthly elements, which moved linearly, *Aither* circulated around heavenly bodies in circular orbits within ethereal spheres. In his *Cratylus*, Plato suggests that *aither* derives from *aei thein* ("always running"). Aither also serves as a backdrop for parts of Giulio Romano's c.1533 *trompe l'oeil* frescoed ceiling in the *Chamber of the Giants* (a portion *above*), Palazzo del Te, Mantua, Italy. Volatile and intoxicating, our earthly anodyne, ether, may have seemed a gift from the gods. Alas, the only "blues" ether elicited in 19th-century mortals arose from recreational overindulgence.

Melissa L. Coleman, M.D., Assistant Professor, Department of Anesthesiology and Perioperative Medicine, Penn State College of Medicine, Hershey, Pennsylvania, and George S. Bause, M.D., M.P.H., Wood Library-Museum Curator Emeritus.

ANESTHESIOLOGY

Intraoperative Hypotension and Acute Kidney Injury, Stroke, and Mortality during and outside Cardiopulmonary Bypass: A Retrospective Observational Cohort Study

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Single-center data demonstrate that intraoperative hypotension during cardiac surgery is independently associated with stroke and acute kidney injury
- The reproducibility of this observation and whether the timing of hypotension during cardiac surgery (within *vs.* outside the cardiopulmonary bypass period) modifies the association remain unclear

What This Article Tells Us That Is New

- Among 4,984 patients undergoing cardiac surgery at a single tertiary care center between 2008 and 2016, 256 (5.1%) experienced the primary outcome of stroke (66, 1.3%), acute kidney injury (125, 2.5%), or mortality (109, 2.2%)
- Each 10 min of hypotension (mean arterial pressure of less than 65 mmHg) during, before, or after cardiopulmonary bypass was associated with an increased odds ratio of 1.06 (95% CI, 1.03 to 1.10; $P = 0.001$)
- Intraoperative hypotension, even if it occurs outside of cardiopulmonary bypass, is independently associated with stroke, acute kidney injury, or death after cardiac surgery

ABSTRACT

Background: In cardiac surgery, the association between hypotension during specific intraoperative phases or vasopressor-inotropes with adverse outcomes remains unclear. This study's hypothesis was that intraoperative hypotension duration throughout the surgery or when separated into hypotension during and outside cardiopulmonary bypass may be associated with postoperative major adverse events.

Methods: This retrospective observational cohort study included data for adults who had cardiac surgery between 2008 and 2016 in a tertiary hospital. Intraoperative hypotension was defined as mean arterial pressure of less than 65 mmHg. The total duration of hypotension was divided into three categories based on the fraction of overall hypotension duration that occurred during cardiopulmonary bypass (more than 80%, 80 to 60%, and less than 60%). The primary outcome was a composite of stroke, acute kidney injury, or mortality during the index hospitalization. The association with the composite outcome was evaluated for duration of hypotension during the entire surgery, outside cardiopulmonary bypass, and during cardiopulmonary bypass and the fraction of hypotension during cardiopulmonary bypass adjusting for vasopressor-inotrope dose, milrinone dose, patient, and surgical factors.

Results: The composite outcome occurred in 256 (5.1%) of 4,984 included patient records; 66 (1.3%) patients suffered stroke, 125 (2.5%) had acute kidney injury, and 109 (2.2%) died. The primary outcome was associated with total duration of hypotension (adjusted odds ratio, 1.05; 95% CI, 1.02 to 1.08; $P = 0.032$), hypotension outside cardiopulmonary bypass (adjusted odds ratio, 1.06; 95% CI, 1.03 to 1.10; $P = 0.001$) per 10-min exposure to mean arterial pressure of less than 65 mmHg, and fraction of hypotension duration during cardiopulmonary bypass of less than 60% (reference greater than 80%; adjusted odds ratio, 1.67; 95% CI, 1.10 to 2.60; $P = 0.019$) but not with each 10-min period hypotension during cardiopulmonary bypass (adjusted odds ratio, 1.04; 95% CI, 0.99 to 1.09; $P = 0.118$), fraction of hypotension during cardiopulmonary bypass of 60 to 80% (adjusted odds ratio, 1.45; 95% CI, 0.97 to 2.23; $P = 0.082$), or total vasopressor-inotrope dose (adjusted odds ratio, 1.00; 95% CI, 1.00 to 1.00; $P = 0.247$).

Conclusions: This study confirms previous single-center findings that intraoperative hypotension throughout cardiac surgery is associated with an increased risk of acute kidney injury, mortality, or stroke.

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Postoperative major adverse events frequently occur after cardiac surgery, especially with an increasing number of older and complex patients presenting for cardiac surgical care.[1,2] In patients having noncardiac surgery, intraoperative hypotension is associated with postoperative acute kidney

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injury (AKI),[3–5] myocardial injury,[4–6] stroke,[7] delirium,[8] and mortality.[9–11] Intraoperative hypotension may also be a modifiable risk factor for major adverse events in patients having cardiac surgery.[3,12–14]

While a mean arterial pressure (MAP) of 65 mmHg has been suggested as a population harm threshold in noncardiac surgery patients,[15] there is no clear consensus regarding an optimal blood pressure intervention threshold during cardiac surgery with cardiopulmonary bypass (CPB).[16] Existing fixed absolute blood pressure values used as lower intervention thresholds during cardiac surgery were chosen based on the principle that cerebral blood flow autoregulation remains functional during CPB.[17,18] However, current evidence suggests that lower limits of autoregulation can vary from 40 to 160 mmHg.[19] Therefore, intraoperative hypotension may be an important modifiable risk factor for major adverse events in patients having cardiac surgery. Recently, intraoperative hypotension (MAP less than 65 mmHg) even for only 11 min during CPB has been shown to increase the risk of stroke.[20]

Clinical observations and hemodynamic monitoring drive various treatments such as crystalloids, colloids, blood products, increasing pump flow, vasopressors, and inotropes during cardiac surgery. The association between organ perfusion and postoperative adverse events is complex but may be better understood when considering intraoperative pharmacologic management in addition to blood pressure. We hypothesized that intraoperative hypotension duration throughout the surgery or when separated into hypotension during and outside CPB may be associated with a composite outcome of postoperative AKI, mortality, and stroke after cardiac surgery.

In this study, we thus aimed to explore the association between (1) intraoperative hypotension (defined as MAP less than 65 mmHg) or (2) the fraction of total intraoperative hypotension occurring during CPB with a composite of three major adverse events (stroke, AKI, and mortality) accounting for intraoperative vasopressor and inotrope dose in patients having cardiac surgery with CPB.

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Materials and Methods

Study Design and Participants

In this retrospective observational cohort study, we analyzed the data that were prospectively collected in patients having cardiac surgery from institutional electronic medical records, the Society of Thoracic Surgeons (Chicago, Illinois) adult cardiac surgery database, and Anesthesia Information Systems after institutional review board approval (Beth Israel Deaconess Medical Center, Boston, Massachusetts, protocol No. 2020P000074). The institutional review board waived informed consent, and the article adheres to the applicable Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) standards for observational studies.[21] Data for adult patients (greater than 18 yr of age) who had cardiac surgery with CPB between January 2008 and June 2016 were included. Data for patients who underwent emergency cardiac surgery, aortic surgeries, and other procedures, such as atrial fibrillation ablation, pericardial window, maze procedure, left atrial appendage resection, septal myomectomy, and atrial septal defect closure, or invalid, incomplete, or unavailable demographic, baseline, hemodynamic, and outcome data were excluded (fig. 1).

Anesthesia Management

The perioperative details of anesthesia management have been described previously.[22] In brief, preoperative medications were continued until the time of the surgery unless contraindicated by the patient's condition. All patients received a preoperative carotid scan. Anyone with 80% or more carotid stenosis on either side or symptoms suggestive of carotid stenosis was consulted with vascular surgery for appropriate management before surgery. All patients underwent general anesthesia with endotracheal intubation. Standard IV induction was performed using IV propofol and fentanyl. Rocuronium was used for muscle relaxation and isoflurane (0.5 to 1%) for maintenance. Valvular surgeries were performed under cardiac arrest using a standard institutional cardioplegia solution (60 mEq potassium, 8 mEq magnesium, 2.5 g dextrose, 10 mEq Tromethamine, and 500 ml normal saline). The patients were maintained under mild hypothermia, and an α-stat pH strategy was used for blood gas management during CPB.

Hemodynamic Monitoring

All patients received transoesophageal echocardiography (TEE) monitoring throughout the surgery unless contraindicated. The rate, rhythm, preload, afterload, and contractility were maintained with information obtained from arterial catheters, pulmonary artery or central venous catheters, and TEE. All cardiac anesthesiologists involved in patient care were TEE board–certified. After induction, IV phenylephrine or norepinephrine infusions were started to maintain a systolic blood pressure of 90 to 120 mmHg.

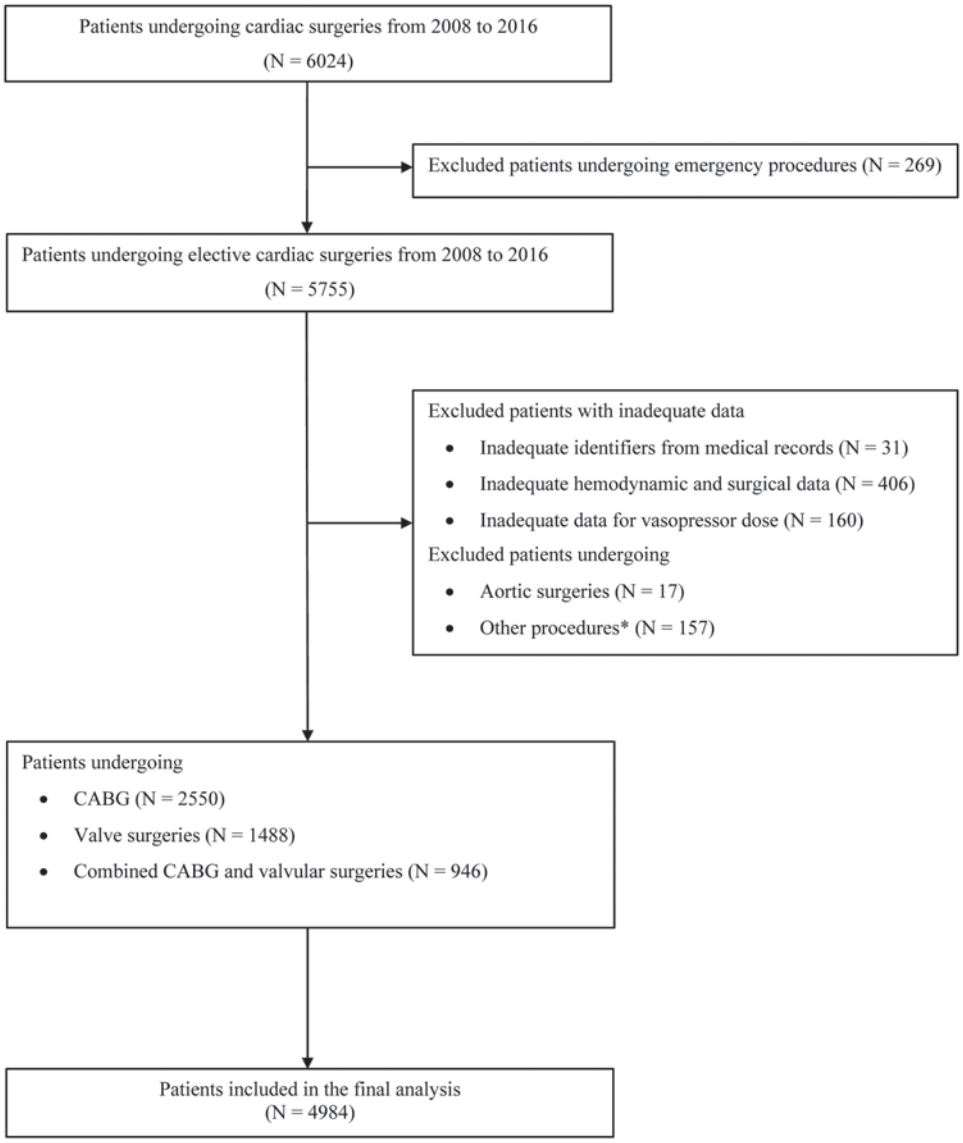

Fig. 1. Flow chart presenting patient selection and analysis. *Other procedures include atrial fibrillation ablation, pericardial window, maze procedure, left atrial appendage resection, septal myomectomy, and atrial septal defect closure. CABG, coronary artery bypass graft.

Vasopressor and Inotrope Treatment

After CPB, in patients with low to moderately dysfunctional ventricular function (preoperative left ventricular ejection fraction less than 30% or with significant mitral or aortic regurgitation), IV epinephrine was started to maintain a cardiac index greater than 2 $l \cdot min^{-1} \cdot m^{-2}$. Intravenous milrinone was added to this regimen to achieve the same goals at the clinician's discretion. Other medications, such as dopamine and dobutamine, were rarely added to this strategy. Afterload was maintained with phenylephrine, norepinephrine, or both, either by themselves or along with the inotropes. The management was guided by direct visualization of the ventricles, echocardiographic assessment of cardiac function with or without pulmonary artery catheter measurements of mixed venous oxygen saturation, and cardiac index.

Vasopressor-inotrope dose was calculated by adding norepinephrine equivalents of total norepinephrine, epinephrine, phenylephrine, and vasopressin dose used during surgery, using the following formula: total vasopressor-inotrope dose = [norepinephrine (μg/min) × min] + [epinephrine (μg/min) × min] + [(phenylephrine (μg/min) × min) ÷ 10] + [vasopressin (U/h) × 8.33 × min].[23,24] The total dose of milrinone used was obtained from the database and analyzed separately.

Definition of Intraoperative Hypotension

Invasive blood pressure was recorded every 15 s, and artifacts were removed using the rules that were previously described.[5,25] Intraoperative hypotension was defined as MAP less than 65 mmHg per the noncardiac surgical literature.[15] It was characterized by (1) total duration as cumulative minutes and (2) area under a MAP of 65 mmHg (area under the receiver operating characteristics curve [AUC]–MAP 65 mmHg) measured based on the trapezoidal rule.[10,26] Using this method, values below the threshold were subtracted from the threshold, multiplied by 0.25 (which corresponds to 15 s), and summed together. For example, each MAP value x less than 65 mmHg will be subtracted from 65, multiplied by 0.25 min, and all such values were summed together.

$$\text{AUC} = \Sigma\left[\left(65 - x\right) \times 0.25\right] \text{ mmHg} \times \text{min}$$

Intraoperative Hypotension during CPB

The duration of intraoperative hypotension was divided into three categories based on how much of the total intraoperative hypotension occurred during CPB (fraction of intraoperative hypotension during CPB): (1) more than 80%, (2) 80 to 60% (inclusive), and (3) less than 60%.

Study Outcome

The primary outcome was a composite of three major adverse events: (1) stroke, defined as any confirmed postoperative neurologic deficit of abrupt onset caused by a disturbance in cerebral blood supply that did not resolve within 24 h; (2) AKI, defined as one or both of the following: threefold rise in serum creatinine from baseline or a creatinine level greater than 4.0 mg/dl with a minimum rise of 0.5 mg/dl and/or a new requirement for dialysis postoperatively; and (3) mortality, defined as all deaths, regardless of cause, occurring during the hospitalization in which the surgery was performed, even if after 30 days (including patients transferred to other acute care facilities) or all deaths, regardless of cause, occurring after discharge from the hospital, but before the end of postoperative day 30, based on Society of Thoracic Surgeons definition, version 2.73 (Supplemental Digital Content 1, appendix 1, http://links.lww.com/ALN/C817). Stroke or AKI occurring during the index hospitalization, even if more than 30 days, was included in the primary outcome.

Statistical Analysis

Descriptive statistics were computed as mean values with SD for continuous variables and frequencies with percentages for categorical variables. The Kruskal–Wallis test was used for normality testing. Continuous (normal and nonnormal) and categorical measures were appropriately tested with t tests, Wilcoxon rank sum tests, and chi-square tests, respectively. Multivariable logistic regression models were used to assess the association between intraoperative hypotension per 10-min exposure to MAP less than 65 mmHg, AUC-MAP 65 mmHg, vasopressor-inotrope dose, total dose of milrinone, and the composite primary outcome (primary model). Confounders were predefined based on clinical plausibility, which included age in years, sex, surgery category, Society of Thoracic Surgeons risk scores in tertiles, preoperative left ventricular ejection fraction, change in hematocrit percentage, and aortic cross-clamp time. The same confounders were used for both intraoperative hypotension variables (total duration per 10-min exposure to MAP less than 65 mmHg and AUC-MAP 65 mmHg) analyses. We introduced the statistically ($P < 0.10$ in the univariable analysis) and clinically significant variables into the model. The total duration of intraoperative hypotension per 10-min exposure to MAP less than 65 mmHg and AUC-MAP 65 mmHg were considered as two separate multivariable analyses. In an attempt to determine the phase-specific associations of intraoperative hypotension during cardiac surgery, we constructed similar multivariable logistic regression models exploring the relationship between hypotension duration per 10-min exposure to MAP less than 65 mmHg during and outside CPB with the composite primary outcome. Similar models were used to explore the association between the fraction of hypotension duration during CPB (greater than 80%, 80 to 60%, and less than 60%) with the composite outcome and individual major adverse events.

The linearity assumption of the effects of intraoperative hypotension, vasopressor-inotrope dose, and total dose of milrinone on the log odds of the outcome variable was assessed using restricted cubic splines with three knots and generalized additive models. In the cases in which significant nonlinear terms were detected, the corresponding variables were categorized using tertiles/quartiles. The effect modification of vasopressor-inotrope dose and total dose of milrinone on the association between intraoperative hypotension and the composite outcome was evaluated by including an interaction term of vasopressor-inotrope dose and intraoperative hypotension in the logistic regression models. In the case of nonsignificant interaction, the final model only with main effects was reported. The Hosmer–Lemeshow goodness-of-fit test and AUC were used to assess the model fit. The precision-recall AUC was reported given the class imbalance in this data set. The variable inflation factor was used to assess the correlation between predictors. Intraoperative hypotension duration was introduced into the model as a restricted cubic spline for measuring the predicted probabilities of the composite primary outcome. Statistical analyses were performed in R version 3.5.1 and RStudio version 1.1.463 (RStudio, USA). All tests were two-sided, with 0.05 as the level of significance. Correction for multiple testing and models was not performed. No specific power calculation was performed for this retrospective

observational cohort study. The data analysis and statistical plan were written after the data were accessed.[27]

Sensitivity Analyses

We performed two sensitivity analyses to further explore the robustness of the association between intraoperative hypotension and the composite primary outcome. The first sensitivity analysis was designed to examine the association between total duration and AUC-MAP 65 mmHg with the composite outcome wherein we used preoperative comorbid conditions as the variables in the multivariable logistic regression models instead of the Society of Thoracic Surgeons risk score. Although we used the Society of Thoracic Surgeons risk score, which itself includes all preoperative comorbidities, in addition to multiple other perioperative variables in our primary analyses, we wanted to examine the association when considering individual comorbidities. The second sensitivity analysis was designed to eliminate the potential impact of outlier cases with an extremely long duration of hypotension. Accordingly, we excluded the top 5% of patients from our final cohort and determined the association between total hypotension duration and AUC-MAP 65 mmHg with the composite outcome. Our primary model in this study evolved as a result of the peer review process, and we focused on specific models following the suggestions from reviewers and editors.

Results

Baseline Characteristics

Figure 1 displays an overview of patient selection and analysis. Data describing 6,024 patients who had cardiac surgeries from 2008 to 2016 were collected. We excluded 269 patients who had emergent surgeries. From the remaining 5,755 patients, 597 patients were excluded due to inadequate data; 174 patients who underwent procedures other than coronary artery bypass graft, valve, or combined were excluded; and the final analysis included 4,984 patients. Table 1 summarizes the baseline characteristics of patients stratified by the postoperative composite outcome. The mean ± SD age of our study population was 67 ± 11 yr. There were 3,491 (70%) male patients, 1,165 (23%) patients had diabetes, 3,933 (79%) patients had hypertension, and 3,807 (76%) patients had dyslipidemia. Previous myocardial infarction was present in 1,565 (31%), congestive heart failure was present in 1,741 (35%), and 85 (2%) patients were on dialysis before surgery.

Postoperative Major Adverse Events

Postoperative major adverse events are displayed in Supplemental Digital Content 2 (table 1, http://links.lww.com/ALN/C818). The composite primary outcome occurred in 256 (5.1%). AKI was present in 125 (2.5%) patients, 66 (1.3%) patients had a stroke, and 109 (2.2%) patients died after surgery. Baseline characteristics of patients stratified by the composite primary outcome are presented in table 1. Patients having one of the major adverse events of the composite primary outcome were significantly older and more likely to be male and have diabetes (table 1). They also had a higher incidence of congestive heart failure, previous myocardial injury, chronic lung disease, and need for inotropic support and steroid medications as compared to those without the composite primary outcome. Society of Thoracic Surgeons risk scores were statistically significantly higher in patients with the composite primary outcome compared to those without (mean ± SD, 0.05 ± 0.06 *vs.* 0.02 ± 0.03; $P < 0.001$).

In addition, patients with the composite primary outcome had a statistically significant increased mean ± SD CPB time (121 ± 61 *vs.* 93 ± 36 min; $P < 0.001$), cross-clamp time (89 ± 42 *vs.* 73 ± 29 min; $P < 0.001$], duration of MAP less than 65 mmHg (143 ± 75 *vs.* 104 ± 48 min; $P < 0.001$) and AUC-MAP 65 mmHg (1,528 ± 1,134 *vs.* 1,070 ± 656 mmHg/min; $P < 0.001$) compared to those without the composite primary outcome. Similarly, median (interquartile range) vasopressor-inotrope dose (milligrams) was statistically significantly higher in patients with the composite primary outcome compared to those without the composite primary outcome (0.98 [0.53 to 2.24] *vs.* 0.65 [0.36 to 1.07] mg; $P < 0.001$; table 1).

Association between Intraoperative Hypotension, Vasopressor-Inotrope Dose, Total Dose of Milrinone, and the Composite Primary Outcome

Every 10-min increase in intraoperative hypotension duration of MAP less than 65 mmHg, AUC-MAP 65 mmHg, vasopressor-inotrope dose, and total dose of milrinone were categorized as continuous variables. The results of multivariable logistic regression analysis evaluating the association between the total duration of intraoperative hypotension throughout the surgery and the composite primary outcome are displayed in table 2. The association of total duration of intraoperative hypotension per 10-min exposure to MAP less than 65 mmHg with the composite primary outcome was statistically significant (adjusted odds ratio, 1.05; 95% CI, 1.02 to 1.08; $P = 0.032$). No statistically significant associations were observed for vasopressor-inotrope dose or the total dose of milrinone (adjusted odds ratio, 1.00; 95% CI, 1.00 to 1.00; $P = 0.247$; and adjusted odds ratio, 1.00; 95% CI, 0 to 1.00; $P = 0.648$).

Statistically significant associations were seen with intraoperative hypotension defined as AUC-MAP 65 mmHg throughout the surgery and the composite outcome (adjusted odds ratio, 1.00; 95% CI, 1.00 to 1.00; $P < 0.001$; Supplemental Digital Content 3, table 2, http://links.lww.com/ALN/C819). However, no statistically significant associations were seen with vasopressor-inotrope dose or total dose of milrinone (adjusted odds ratio, 1.00; 95% CI,

Table 1. Demographics and Clinical Characteristics Stratified by Composite Primary Outcome

Demographics	All Patients (N = 4,984)	Composite Primary Outcome*		*P* Value†
		Absent (N = 4,728 [94.8])	Present (N = 256 [5.1])	
Age, yr, mean ± SD	67 ± 12	67 ± 12	71 ± 12	< 0.001
Male	3,491 (70)	3,329 (70)	162 (63)	0.019
Diabetes	1,165 (23)	1,090 (23)	75 (29)	0.026
Dyslipidemia	3,807 (76)	3,606 (76)	201 (79)	0.454
Hypertension	3,933 (79)	3,713 (79)	220 (86)	0.006
Smoking	1,435 (29)	1,357 (29)	78 (31)	0.591
Congestive heart failure	1,741 (35)	1,590 (34)	151 (59)	< 0.001
Previous myocardial infarction	1,565 (31)	1,458 (31)	107 (42)	< 0.001
Chronic lung disease	596 (12)	547 (12)	49 (19)	< 0.001
Dialysis	85 (2)	79 (2)	6 (2)	0.574
Ejection fraction	54.08 (14)	54.17 (14)	52.45 (15)	0.050
Preoperative medications				
β-Blockers	3,741 (75)	3,554 (75)	187 (73)	0.490
Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers	2,195 (44)	2,085 (44)	110 (43)	0.772
Inotropes	44 (0.9)	32 (0.7)	12 (4.7)	< 0.001
Steroids	168 (3.4)	149 (3.2)	19 (7.4)	< 0.001
Aspirin	4,147 (83)	3,942 (83)	205 (80)	0.197
Statins	3,886 (78)	3,689 (78)	197 (77)	0.745
Society of Thoracic Surgeons risk score, mean ± SD	0.02 ± 0.03	0.02 ± 0.03	0.05 ± 0.06	< 0.001
Surgery category				< 0.001
Coronary artery bypass grafting	2,550 (51)	2,466 (52)	84 (33)	
Coronary artery bypass grafting and valve	946 (19)	854 (18)	92 (36)	
Valve	1,488 (30)	1,408 (30)	80 (31)	
Duration of phase, min, mean ± SD				
Cardiopulmonary bypass	95 ± 38	93 ± 36	121 ± 61	< 0.001
Aortic cross-clamp time	74 ± 30	73 ± 29	89 ± 42	< 0.001
Hematocrit change, mean ± SD	2.77 ± 8	2.82 ± 8	1.84 ± 8	0.066
Colloids, ml, mean ± SD	474 ± 181	472 ± 188	500 ± 0	0.839
Crystalloids, ml, mean ± SD	2,957 ± 3,313	2,951 ± 3,391	3,081 ± 1,045	0.545
Duration MAP less than 65 mmHg, min, mean ± SD	106 ± 51	104 ± 48	143 ± 75.34	< 0.001
Area under the curve–MAP less than 65 mmHg, mmHg · min, mean ± SD	1,094 ± 696	1,070 ± 656	1,528 ± 1,135	< 0.001
Vasopressor-inotrope dose (median), mg [interquartile range]	0.66 [0.37–1.10]	0.65 [0.36–1.07]	0.98 [0.53–2.24]	< 0.001
Milrinone dose (median), mg [interquartile range]	0.00 [0.00–0.00]	0.00 [0.00–0.00]	0.00 [0.00–0.00]	< 0.001

The data are presented as numbers (percentages) unless otherwise indicated.

*Composites of acute kidney injury, stroke, and mortality. †*P* values from chi-square test, Student's *t* test, or Wilcoxon rank sum test, as appropriate.

MAP, mean arterial pressure.

1.00 to 1.00; $P = 0.475$; and adjusted odds ratio, 1.00; 95% CI, 0 to 1.00; $P = 0.756$; Supplemental Digital Content 3, table 2, http://links.lww.com/ALN/C819).

The relationship between the total duration of intraoperative hypotension (quartiles), vasopressor-inotrope dose (quartiles), and the composite primary outcome rate is shown in figure 2. A higher total duration of hypotension and vasopressor-inotrope dose was associated with a higher composite primary outcome incidence (fig. 2). While assessing the model fit, the models demonstrated an average AUC of 0.761 and precision-recall AUC of 0.164.

Association between Hypotension during CPB Phase, outside CPB Phase, and the Composite Primary Outcome

Tables 3 and 4 illustrate the results of multivariable logistic regression models examining the association between duration of hypotension per 10-min exposure to MAP less than 65 mmHg during and outside the CPB phase with the composite primary outcome. Statistically significant associations were seen with hypotension per 10-min exposure to MAP less than 65 mmHg (adjusted odds ratio, 1.06; 95% CI, 1.03 to 1.10; $P = 0.001$) outside the CPB phase and the composite outcome. However, hypotension per 10-min exposure to MAP less than 65 mmHg during CPB phase (adjusted odds ratio, 1.04; 95% CI, 0.99 to 1.09; $P = 0.118$) was not associated with the primary outcome.

Association between the Fraction of Hypotension during CPB and the Composite Primary Outcome

The association between varying fractions of hypotension during CPB and the composite primary outcome is presented in table 5. When compared with more than 80% hypotension duration occurring during CPB, exposure

Table 2. Association between Total Duration of Intraoperative Hypotension per 10-min Exposure to MAP Less than 65 mmHg, Vasopressor-Inotrope Dose, Milrinone Dose, and Composite Primary Outcome

Variables	Odds Ratio	95% CI*	*P* Value
Age	1.00	0.99–1.02	0.699
Female	1.04	0.78–1.39	0.851
Category			
CABG and valve	1.10	0.77–1.61	0.521
Valve	1.05	0.74–1.50	0.730
Change in hematocrit	0.99	0.97–1.00	0.101
Ejection fraction	1.00	0.99–1.01	0.815
Duration of surgery	1.04	1.01–1.07	< 0.001
Aortic cross-clamp time	1.00	0.99–1.00	0.295
Society of Thoracic Surgeons risk score†			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.62	1.00–2.69	0.046
Tertile 3	5.05	3.06–8.50	< 0.001
MAP less than 65 mmHg per 10 min	1.05	1.02–1.08	0.032
Vasopressor-inotrope dose	1.00	1.00–1.00	0.247
Milrinone dose	1.00	0.00–1.00	0.648

*Composite of acute kidney injury, stroke, and mortality adjusted for age, sex, type of surgery, Society of Thoracic Surgeons risk score, left ventricular ejection fraction, Change in hematocrit percentage, duration of surgery, and cross-clamp time. †Society of Thoracic Surgeons risk score categorized into tertiles: less than 0.01, 0.01 to 0.02, and greater than 0.02 to 0.52.

CABG, coronary artery bypass grafting; MAP, mean arterial pressure.

to 80 to 60% of hypotension during CPB was not statistically significantly associated with the primary composite outcome (adjusted odds ratio, 1.45; 95% CI, 0.97 to 2.23; $P = 0.082$), while less than 60% of hypotension occurring during CPB was statistically significantly associated with the primary composite outcome (odds ratio, 1.67; 95% CI, 1.10 to 2.60; $P = 0.019$). The associations between varying fractions of hypotension occurring during CPB with individual components of the composite outcome are presented in Supplemental Digital Content 4 (table 3, http://links.lww.com/ALN/C820).

Sensitivity Analyses

The association between the total duration of hypotension per 10-min exposure to MAP less than 65 mmHg and AUC-MAP 65 mmHg with the composite primary outcome remained statistically significant in sensitivity analyses. In the model that included preoperative comorbidities instead of the Society of Thoracic Surgeons risk score (Supplemental Digital Content 5, table 4, http://links.lww.com/ALN/C821), both duration and AUC-MAP 65 mmHg were statistically significantly associated with the composite primary outcome (adjusted odds ratio, 1.00; 95% CI, 1.00 to 1.01; $P = 0.002$; and adjusted odds ratio, 1.00; 95% CI, 1.00 to 1.00; $P = 0.017$). Similarly, in the other sensitivity analysis that explored similar associations excluding the top 5% of patients who had an extremely long duration of hypotension (Supplemental Digital Content 6, table 5, http://links.lww.com/ALN/C822), the relationship between duration and AUC-MAP 65 mmHg was similar to our primary analysis (adjusted odds ratio, 1.01; 95% CI, 1.00 to 1.01; $P < 0.001$; and adjusted odds ratio, 1.00; 95% CI, 1.00 to 1.00; $P = 0.002$).

Missing Data

Supplemental Digital Content 7 (table 6, http://links.lww.com/ALN/C823) illustrates the results of demographic information comparing patients included in the final cohort and patients who were excluded due to missing data.

Predicted Probabilities of the Composite Primary Outcome

Supplemental Digital Content 8 (fig. 1, http://links.lww.com/ALN/C824) presents the predicted probabilities of the composite primary outcome plotted against the total duration of intraoperative hypotension. The top 5% of the cohort have extremely long durations of hypotension (approximately 200 min; Supplemental Digital Content 8, fig. 1A, http://links.lww.com/ALN/C824). Supplemental Digital Content 8 (fig. 1B, http://links.lww.com/ALN/C824) presents a similar relationship excluding this top 5% of patients from the final cohort. Supplemental Digital Content 9 (fig. 2, http://links.lww.com/ALN/C825) illustrates the predicted probabilities of the composite primary outcome with vasopressor-inotrope dose (Supplemental Digital Content 9, fig. 2A) and the total dose of milrinone (Supplemental Digital Content 9, fig. 2B).

Supplemental Digital Content 10 (fig. 3, http://links.lww.com/ALN/C826) demonstrates the distribution of the Society of Thoracic Surgeons risk score among the final cohort of patients. We could observe the nonlinearity of the Society of Thoracic Surgeons risk score among the cohort.

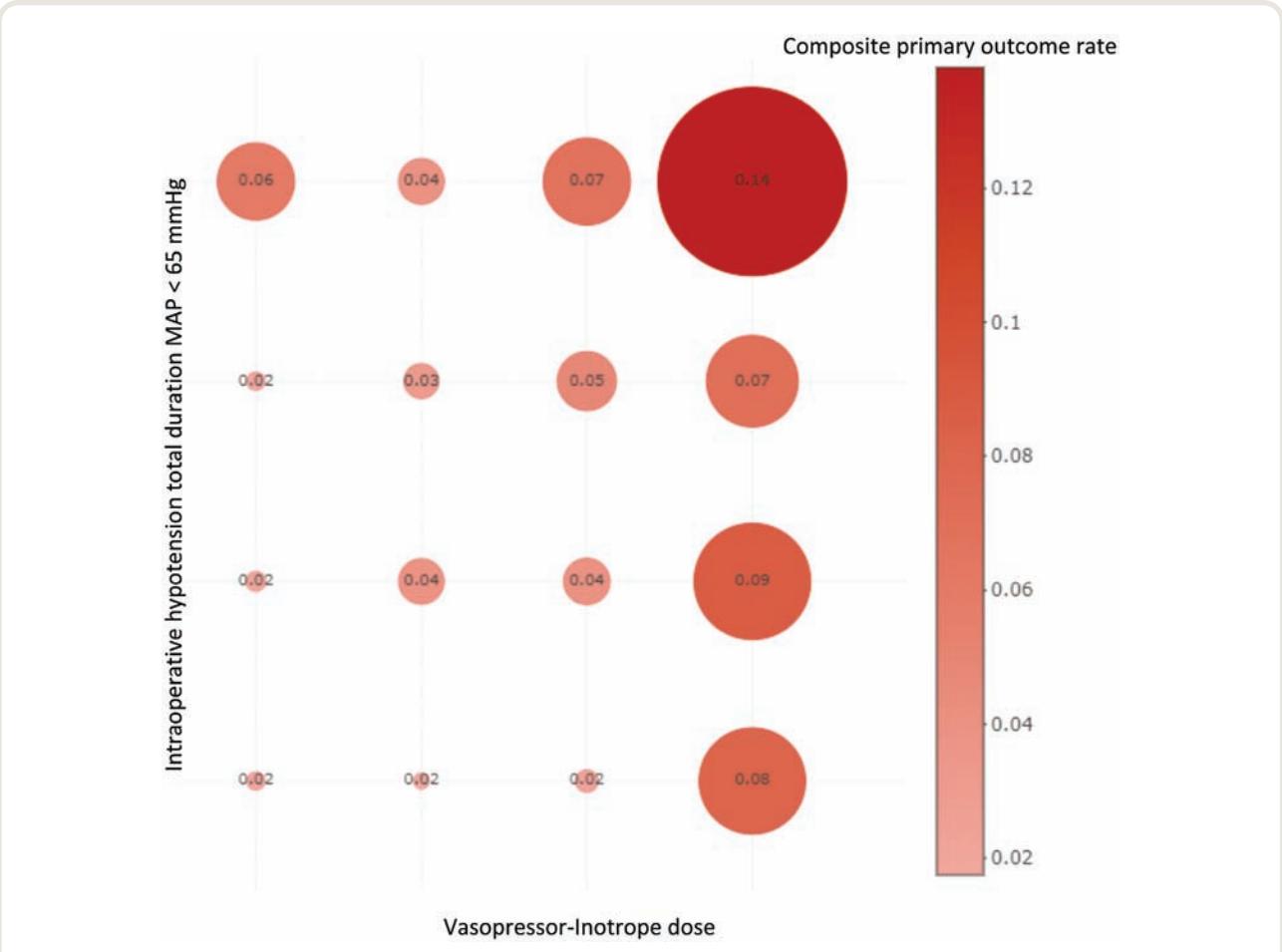

Fig. 2. Association between total duration of intraoperative hypotension mean arterial pressure (MAP) less than 65 mmHg, vasopressor-inotrope dose, and composite primary outcome. The *x axis* corresponds to vasopressor-inotrope dose in quartiles, whereas the *y axis* corresponds to the total duration of intraoperative hypotension MAP less than 65 mmHg in quartiles. *Bubble size* and *color* represent composite primary outcome rate.

Supplemental Digital Content 11 (fig. 4, http://links.lww.com/ALN/C827) illustrates the correlation between intraoperative hypotension total duration with vasopressor-inotrope dose, total dose of milrinone, Society of Thoracic Surgeons risk score, age, sex, surgery category, hematocrit change, ejection fraction, duration of surgery, and cross-clamp time. We provide open access to all our data extraction, filtering, data wrangling, modeling, figures, and table code and queries at https://github.com/theonesp/vasopressor_dose_mae.

Discussion

In patients undergoing cardiac surgery with CPB, the total duration of intraoperative hypotension per 10-min exposure of MAP less than 65 mmHg throughout the surgery was statistically significantly associated with the composite primary outcome of stroke, AKI, or death. Similar associations were seen with the duration of intraoperative hypotension outside the CPB phase and composite primary outcome.

We observed a significant association between intraoperative hypotension during cardiac surgery and adverse outcomes. These results broadly support the work of previous research showing that hypotension during cardiac surgery was significantly associated with stroke,[20] renal injury,[28] and mortality.[29] Similar to this research, in studies exploring the CPB phase–specific hypotension in patients undergoing cardiac surgery, the risk of postoperative stroke[20] and renal replacement therapy[28] increased for every 10-min exposure to MAP less than 65 mmHg.

In addition to duration, we characterized the severity of intraoperative hypotension as AUC-MAP 65 mmHg. Both total intraoperative hypotension duration and AUC-MAP 65 mmHg showed similar results. Our results were consistent with previous studies,[5,25] in which both the duration and AUC for blood pressure thresholds were associated with adverse outcomes.

Table 3. Association between Intraoperative Hypotension per 10-min Exposure to MAP Less than 65 mmHg during CPB Phase, Vasopressor-Inotrope Dose, Milrinone Dose, and Composite Primary Outcome

Variables	Odds Ratio	95% CI*	*P* Value
Age	1.00	0.99–1.02	0.679
Female	1.02	0.77–1.37	0.846
Category			
CABG and valve	1.12	0.77–1.63	0.545
Valve	1.09	0.77–1.55	0.636
Change in hematocrit	0.98	0.97–1.00	0.080
Ejection fraction	1.00	0.99–1.01	0.780
Duration of surgery	1.05	1.03–1.08	< 0.001
Aortic cross-clamp time	1.00	0.99–1.00	0.171
Society of Thoracic Surgeons risk score†			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.67	1.03–2.76	0.042
Tertile 3	5.40	3.29–9.06	< 0.001
MAP less than 65 mmHg during CPB phase per 10 min	1.04	0.99–1.09	0.118
Vasopressor-inotrope dose	1.00	1.00–1.00	0.243
Milrinone dose	1.00	0.00–1.00	0.632

*Composite of acute kidney injury, stroke, and mortality adjusted for age, sex, type of surgery, Society of Thoracic Surgeons risk score, left ventricular ejection fraction, Change in hematocrit percentage, duration of surgery, and cross-clamp time. †Society of Thoracic Surgeons risk score categorized into tertiles: less than 0.01, 0.01 to 0.02, and greater than 0.02 to 0.52.
CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; MAP, mean arterial pressure.

Table 4. Association between Intraoperative Hypotension per 10-min Exposure to MAP Less than 65 mmHg, Outside CPB Phase, Vasopressor-Inotrope Dose, Milrinone Dose, and Composite Primary Outcome

Variables	Odds Ratio	95% CI*	*P* Value
Age	1.00	0.99–1.01	0.843
Female	1.01	0.76–1.34	0.940
Category			
CABG and valve	1.12	0.77–1.62	0.567
Valve	1.09	0.77–1.54	0.637
Change in hematocrit	0.99	0.97–1.00	0.117
Ejection fraction	1.00	0.99–1.01	0.798
Duration of surgery	1.05	1.02–1.07	< 0.001
Aortic cross-clamp time	1.00	0.99–1.00	0.703
Society of Thoracic Surgeons risk score†			
Tertile 1	Reference	Reference	
Tertile 2	1.62	0.99–1.01	0.057
Tertile 3	5.00	3.03–8.42	< 0.001
MAP less than 65 mmHg outside CPB phase per 10 min	1.06	1.03–1.10	0.001
Vasopressor-inotrope dose	1.00	1.00–1.00	0.235
Milrinone dose	1.00	0.00–1.00	0.645

*Composite of acute kidney injury, stroke and mortality adjusted for age, sex, type of surgery, Society of Thoracic Surgeons risk score, left ventricular ejection fraction, Change in hematocrit percentage, duration of surgery, and cross-clamp time. †Society of Thoracic Surgeons risk score categorized into tertiles: less than 0.01, 0.01 to 0.02, and greater than 0.02 to 0.52.
CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; MAP, mean arterial pressure.

When exploring an intraoperative phase–specific relationship with adverse events, hypotension duration outside the CPB phase was significantly associated with the primary outcome. Our results mirror the findings of a similar study in which MAP less than 65 mmHg for 10 min or more during the post-CPB phase significantly increased the risk of adverse events.[28] Compared with the fraction of overall hypotension duration occurring during CPB of more than 80%, a fraction of hypotension duration occurring during CPB of less than 60% was statistically significantly associated with a higher risk of the composite outcome. This observation may be explained by the physiologic stress during the post-CPB phase. Various mechanisms such as catecholamine surges, mechanical trauma to red blood cells triggering inflammatory states, and decreased vasomotor reactivity after CPB in the presence of hypotension could worsen end-organ damage, resulting in adverse outcomes. It is possible, therefore, that hypotension outside the CPB

Table 5. Association between Fraction of Intraoperative Hypotension Duration during CPB and Composite Primary Outcome

Variables	Odds Ratio	95% CI*	*P* Value
Age	1.00	0.99–1.02	0.782
Female	1.01	0.75–1.33	0.972
Category			
CABG and valve	1.16	0.80–1.70	0.431
Valve	1.19	0.84–1.68	0.324
Change in hematocrit	0.98	0.97–1.00	0.081
Ejection fraction	1.00	1.00–1.01	0.971
Duration of surgery	1.01	1.00–1.01	< 0.001
Aortic cross-clamp time	1.00	0.99–1.01	0.972
Society of Thoracic Surgeons risk score†			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.56	0.97–2.55	0.069
Tertile 3	4.41	2.70–7.35	< 0.001
Fraction of intraoperative hypotension duration during CPB (MAP less than 65 mmHg)			
80 to 60%	1.45	0.97–2.23	0.082
Less than 60%	1.67	1.10–2.60	0.019
Vasopressor-inotrope dose	1.00	1.00–1.00	0.247
Milrinone dose	1.00	0–1.00	0.639

*Composite of acute kidney injury, stroke, and mortality adjusted for age, sex, type of surgery, Society of Thoracic Surgeons risk score, left ventricular ejection fraction, Change in hematocrit percentage, duration of surgery, and aortic cross-clamp time. †Society of Thoracic Surgeons risk score categorized into tertiles: less than 0.01, 0.01 to 0.02, and greater than 0.02.
CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; MAP, mean arterial pressure.

phase is an important risk factor that needs to be taken into consideration, and more research is needed regarding the effects of hypotension during and outside CPB.[30,31]

Advanced hemodynamic monitoring and protocolized treatment strategies in the cardiac surgical setting can identify and treat hemodynamic changes earlier than in noncardiac surgery with noninvasive monitoring. In this study, we did not observe a statistically significant association between vasopressor-inotrope dose or total dose of milrinone and the composite outcome. Even though vasopressor-inotropes were used to treat hypotension using advanced hemodynamic monitoring, the duration of intraoperative hypotension was still associated with the composite outcome.

Transesophageal echocardiography, invasive arterial blood pressure monitoring, and central venous pressures were routinely used in our care setting to optimize fluids and maintain blood pressures throughout cardiac surgery. In this context, vasopressor use to optimize blood pressures was not associated with a higher or lower incidence of major adverse events in our study. Other studies have shown a similar lack of reduction in postoperative adverse outcomes with the use of vasopressors to achieve higher MAP thresholds.[22,32–34] It remains to be explored whether vasopressor treatment aiming to achieve a fixed intraoperative blood pressure target (*e.g.*, MAP of 65 mmHg) could improve postoperative outcomes.

Weis *et al.*[35] observed that cardiac surgical patients needing more vasopressors had a greater degree of a systemic inflammatory response that has been associated with poor outcomes.[36] Moreover, significant associations between increased vasopressor support and renal replacement therapy, length of stay, and periods of ventilation after cardiac surgery were reported.[35]

The relationship between organ perfusion and adverse outcomes remains complex and has recently gained increased scientific attention. Further work is needed to gain a better understanding regarding markers such as oxygen delivery as a measure for tissue perfusion.

This is a retrospective study, and finding a causative link between intraoperative hypotension or vasopressor-inotrope dose and postoperative outcomes is thus not possible. Moreover, the vasopressor-inotrope dose was calculated by adding norepinephrine equivalents of vasopressors and inotropes used. As each drug might have a different molecular target, there is a possibility that we might have missed the effect of an individual medication. The sample size may be relatively small to evaluate the true interaction between intraoperative hypotension, vasopressor-inotrope dose, and outcome. Our analyses did not include data reflecting postoperative hypotension and other factors related to tissue perfusion, such as mixed venous oxygen saturation or blood gas parameters that might have an association with the incidence of adverse outcomes. In addition, our analysis is limited by a possible lack of power for many of the nonsignificant observations and lack of adjustment for multiple testing.

To conclude, in patients having cardiac surgery with CPB, the total duration of intraoperative hypotension per 10-min exposure of MAP less than 65 mmHg was statistically significantly associated with the composite primary outcome of stroke, AKI, or mortality. Hypotension per 10-min exposure of MAP less than 65 mmHg outside the CPB phase was statistically significantly associated with the composite outcome. These results confirm findings from previous studies exploring the intraoperative phase–specific

association between hypotension and adverse outcomes. Vasopressor-inotrope dose or total dose of milrinone was not statistically significantly associated with the composite primary outcome.

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Competing Interests

Dr. Saugel has received honoraria for consulting, honoraria for giving lectures, and refunds of travel expenses from Edwards Lifesciences Inc. (Irvine, California). He has received honoraria for consulting, institutional restricted research grants, honoraria for giving lectures, and refunds of travel expenses from Pulsion Medical Systems SE (Feldkirchen, Germany). Dr. Saugel has received institutional restricted research grants, honoraria for giving lectures, and refunds of travel expenses from CNSystems Medizintechnik GmbH (Graz, Austria). He has received institutional restricted research grants from Retia Medical LLC. (Valhalla, New York). He has received honoraria for giving lectures from Philips Medizin Systeme Böblingen GmbH (Böblingen, Germany). Dr. Saugel has received honoraria for consulting, institutional restricted research grants, and refunds of travel expenses from Tensys Medical Inc. (San Diego, California). The other authors declare no competing interests.

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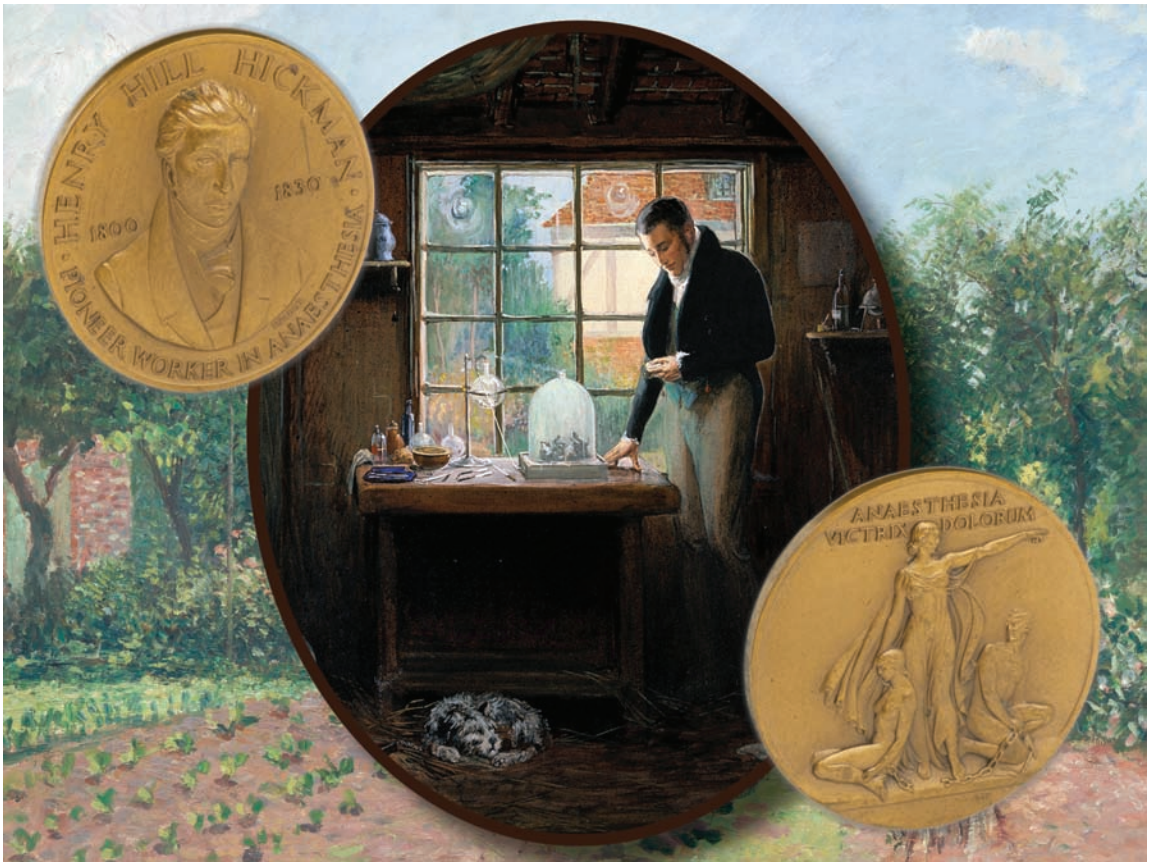
ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Only Half Dead: Henry H. Hickman and "Suspended Animation"



Appalled by the plight of surgical patients before the advent of ether anesthesia, English physician Henry Hill Hickman (1800 to 1830, *center*) championed "suspended animation" as an anesthetic technique. He induced hypercapnic narcosis and transient respiratory arrest by enclosing puppies in glass chambers (*center*). At times, he infused supplemental carbon dioxide to expedite the process. Thereby sedated, the canines would tolerate the removal of parts of their ears without associated pain, hemorrhage, or inflammation. By contrast, the same procedure performed on awake dogs would cause discomfort, bleeding, and edema. Eager to disseminate his findings, Hickman sought the patronage of scientist T. A. Knight of England and King Charles X of France, to no avail. During his short lifetime, no scientific community would endorse his use of controlled asphyxia, which sometimes required rescue insufflation. However, a century after his death, Hickman would be immortalized. Seeking to raise the profile of a burgeoning specialty, the Anaesthetic Section of the Royal Society of Medicine would endow an international honor in his name. Since 1935, the Hickman Medal has been given every 3 years to a physician or scientist with outstanding contributions to anesthesia. The one pictured above, with an image of Hickman on its obverse (*upper left*), and a woman depicting "*Anaesthesia, Victrix Dolorum*" on its reverse (*lower right*), was awarded to Ralph Waters, M.D. (1883 to 1979), pioneer of American academic anesthesiology. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology. www.woodlibrarymuseum.org)

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ANESTHESIOLOGY

Referral Indications for Malignant Hyperthermia Susceptibility Diagnostics in Patients without Adverse Anesthetic Events in the Era of Next-generation Sequencing

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ANESTHESIOLOGY 2022; 136:940–53

EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Most cases of malignant hyperthermia susceptibility are associated with variants in the gene encoding the skeletal muscle ryanodine receptor 1, *RYR1*
- Next-generation sequencing has resulted in a rapid increase in the identification of both the number of patients with an *RYR1* variant and the number of newly identified *RYR1* variants

What This Article Tells Us That Is New

- The hypothesis that there is an increased referral to malignant hyperthermia units of patients without a personal or family history of adverse anesthetic events suspected to be malignant hyperthermia was tested in a retrospective multicenter cohort study
- The proportion of patients referred without a personal or family history of adverse anesthetic events increased from 28.4% (61 of 215) between 2010 and 2014 to 43.6% (133 of 305) between 2015 and 2019
- Patients with a personal or family history of adverse anesthetic events were more frequently diagnosed as malignant hyperthermia–susceptible (133 of 220; 60.5%) than those without (47 of 120; 39.2%)

ABSTRACT

Background: The introduction of next-generation sequencing into the diagnosis of neuromuscular disorders has resulted in an increased number of newly identified *RYR1* variants. The hypothesis was that there is an increased referral of patients to malignant hyperthermia units without a personal/family history of adverse anesthetic events suspected to be malignant hyperthermia. This retrospective multicenter cohort study evaluates patient referral indications and outcomes for those without a history of an adverse anesthetic event.

Methods: Patients referred between 2010 and 2019 to the malignant hyperthermia units in Antwerp, Belgium; Lund, Sweden; Nijmegen, The Netherlands; and Toronto, Ontario, Canada were included. Previously tested patients and relatives of previously tested patients were excluded. Data collection included demographics, referral details, muscle contracture, and genetic testing results including Rare Exome Variant Ensemble Learner scores. Referral indications were categorized into those with a personal/family history of adverse anesthetic event and other indications including exertional and/or recurrent rhabdomyolysis, *RYR1* variant(s) detected in diagnostic testing in the neuromuscular clinic without a specific diagnosis (in a family member), diagnosed *RYR1*-related myopathy (in a family member), idiopathically elevated resting creatine kinase values, exertional heat stroke, and other.

Results: A total of 520 medical records were included, with the three most frequent referral indications as follows: personal history of an adverse anesthetic event (211 of 520; 40.6%), family history of an adverse anesthetic event (115 of 520; 22.1%), and exertional and/or recurrent rhabdomyolysis (46 of 520; 8.8%). The proportion of patients referred without a personal/family history of an adverse anesthetic event increased to 43.6% (133 of 305) between 2015 and 2019 compared to 28.4% (61 of 215) in 2010 to 2014 ($P < 0.001$). Patients with a personal/family history of an adverse anesthetic event were more frequently diagnosed as malignant hyperthermia–susceptible (133 of 220; 60.5%) than those without (47 of 120; 39.2%; $P < 0.001$). Due to missing data, 180 medical records were excluded.

Conclusions: The proportion of patients referred to malignant hyperthermia units without a personal/family history of an adverse anesthetic event has increased, with 39.2% (47 of 120) diagnosed as malignant hyperthermia–susceptible.

(ANESTHESIOLOGY 2022; 136:940–53)

Malignant hyperthermia (MH) is a potentially life-threatening pharmacogenetic disorder triggered by volatile anesthetics and/or depolarizing muscle relaxants in MH-susceptible individuals. MH susceptibility diagnostics rely on the caffeine–halothane contracture test (CHCT)[1,2] or the *in vitro* contracture test[3] on freshly biopsied muscle tissue and on genotyping.[4] In the majority of cases, MH susceptibility is associated with variants in

This article is featured in "This Month in Anesthesiology," page A1. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). This article has a visual abstract available in the online version. Part of the work presented in this article has been presented at the European Malignant Hyperthermia Group 39th Annual Meeting 2021 (May 14, 2021, virtual meeting), where the presentation was granted the European Malignant Hyperthermia Group Best Clinical Research Presentation Award; at the European Neuromuscular Center 259th International Workshop: Anesthesia and Neuromuscular Disorders (May 29, 2021, virtual meeting); and at the Annual Science Congress from the Dutch Association of Anesthesiologists (October 1, 2021, Ede, The Netherlands), where the presentation was granted the Best Scientific Lecture Award. S.R. and M.M.J.S. contributed equally to this article.

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RYR1, the gene encoding skeletal muscle ryanodine receptor 1.[5] Other MH-associated genes are *CACNA1S*, which encodes the α1 subunit of the dihydropyridine receptor,[6] and *STAC3*, which encodes the SH3 and cysteine-rich domain 3 proteins.[7]

Next-generation sequencing, recently introduced in most institutions in the Western world, facilitates faster, cheaper, and more accurate genetic analysis and has caused a significant paradigm shift in MH susceptibility diagnostics. Furthermore, as *RYR1* variants may cause a wide spectrum of muscle diseases,[8] next-generation sequencing is frequently used in the neuromuscular clinic for *RYR1* analysis in patients with an unresolved neuromuscular phenotype. This has resulted in a considerable rise in the number of newly identified *RYR1* variants,[9] as well as an increased number of referred patients from neuromuscular clinics to MH units to assess the potential risk of MH in patients with an *RYR1* variant of unknown significance, even though they have no personal or family history of adverse anesthetic events suspected to be MH.

For genetic variants to be used in diagnosing MH susceptibility, they need to be classified according to the ClinGen Variant Curation Expert Panel recommendations for *RYR1* pathogenicity classification[10] and/or the European Malignant Hyperthermia Group scoring matrix for classification of genetic variants in MH susceptibility (https://www.emhg.org/genetic-scoring-matrix). If a patient carries a variant that does not meet the criteria of being benign or pathogenic, a CHCT/*in vitro* contracture test is the only option to confirm or rule out MH susceptibility.[4] As currently only a minority of the *RYR1* variants have been classified as benign or pathogenic, MH diagnostics still relies on the CHCT/*in vitro* contracture test. Hence, counseling for MH susceptibility in patients with one or more *RYR1* variants of unknown significance without a personal or family history of adverse anesthetic events suspected to be MH can be challenging, and performing a CHCT/*in vitro* contracture test in all these patients might result in unnecessary invasive muscle biopsies.

We hypothesize that there is an increased referral of patients to MH units without a personal or family history of adverse anesthetic events suspected to be MH. This retrospective multicenter cohort study aims to evaluate the overall referral indications and the results of MH susceptibility diagnostics in patients without a history of adverse anesthetic events suspected to be MH. The knowledge obtained from this study can be used to improve counseling of patients referred to MH centers without a personal or family history of adverse anesthetic events suspected to be MH and to reassess guidelines to test for MH susceptibility.[4]

Materials and Methods

This study was performed with approval of the Research Ethics Boards from the Canisius Wilhelmina Hospital, Nijmegen, The Netherlands (registration No. 067-2020, date of approval September 8, 2020); Skane University Hospital, Lund, Sweden (registration No. 2019-03960, date of approval October 9, 2019); Toronto General Hospital, Toronto, Ontario, Canada (registration No. 19-5365, date of approval May 12, 2019); and Antwerp University Hospital/University of Antwerp, Antwerp, Belgium (registration No. 1805016N, date of approval September 28, 2015). Informed consent was waived due to the retrospective nature of the study.

Study Design

This retrospective multicenter cohort study focusing on the referral indications for MH susceptibility diagnostics consists of two parts in line with the two aims of the study. The primary analysis is a retrospective evaluation of the referral indications and the use of next-generation sequencing during the study period. The secondary analysis is a detailed evaluation of the MH diagnostics performed and the test results of patients referred to the participating MH units without a personal or family history of an adverse anesthetic event suspected to be MH.

Study Population

The medical records of patients referred to one North American (Toronto, Ontario, Canada) and three European (Antwerp, Belgium; Lund, Sweden; and Nijmegen, The

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Netherlands) MH investigation units were evaluated. All medical records of patients referred to the participating centers between January 1, 2010, and December 31, 2019, were reviewed. Medical records of the following patients were excluded from the primary analysis to prevent ascertainment bias:

- Relatives of previously tested patients
- Patients who underwent MH susceptibility diagnostics before 2010 and were referred again for genetic testing to facilitate MH susceptibility diagnostics by genotyping in family members

Medical records of the following patients were excluded from the secondary analysis:

- Patients referred because of a personal or family history of adverse anesthetic event suspected to be MH
- Patients referred because of a referral indication categorized as other (see Referral Indication section)
- Patients genetically investigated using a targeted technique (*e.g.*, targeted screening for pathogenic *RYR1* variants or a hotspot technique), except when referred because of a family history of a diagnosed *RYR1*-related myopathy or a family history of an *RYR1* variant detected in diagnostic testing in the neuromuscular clinic without a specific clinical or histopathologic diagnosis; these patients were parents of neuromuscular patients who were too young to be investigated by a CHCT/*in vitro* contracture test and were genetically investigated utilizing a targeted technique to identify which family members are carriers of the *RYR1* variant(s) identified in the neuromuscular patient.

The study design and the selection process of medical records for the primary and secondary analysis are summarized in figure 1. Some of the medical records included in this study cohort have been described before.[8,11–16] Details of the overlapping medical records are summarized in Supplemental Digital Content 1 (http://links.lww.com/ALN/C833).

Data Collection

The collected data included demographic characteristics (age at referral and sex), referral details (date of referral, indication for referral, and the indication for *RYR1*, *CACNA1S*, or *STAC3* sequencing), clinical grading scale[17] for the referred probands, resting creatine kinase levels, details on the performed genetic tests (genes analyzed, technique used, and test results), and CHCT/*in vitro* contracture test results.

Referral Indication

Referral indications were categorized into two main groups: those with a personal or family history of adverse anesthetic events suspected to be MH and those without. Since our objective was to improve counseling for patients without a history of adverse anesthetic events, referral indications from the latter category were subcategorized into different groups.

A personal or family history of adverse anesthetic events suspected to be MH was defined as follows:

1. A personal history of an adverse anesthetic event suspected to be MH (probands)
2. A family history of an adverse anesthetic event suspected to be MH (relatives who were investigated instead of the proband)

Other referral indications without a history of adverse anesthetic events suspected to be MH were defined as follows:

3. Personal history of exertional and/or recurrent rhabdomyolysis, defined as a creatine kinase value during the rhabdomyolysis event of more than 10,000 U/l, where recurrent is defined as at least two episodes of rhabdomyolysis
4. Personal history of a diagnosed *RYR1*-related myopathy (central core disease, multiminicore disease, centronuclear myopathy, congenital fiber type disproportion, King–Denborough syndrome, periodic paralysis, and axial myopathy)
5. Family history of a diagnosed *RYR1*-related myopathy
6. Personal history of an *RYR1* variant detected on diagnostic testing in the neuromuscular clinic for symptoms suggestive of a neuromuscular disorder but without a specific clinical or histopathologic diagnosis and/or fulfilling one of the other referral criteria; these are often coincidentally found *RYR1* variants; frequent reasons for *RYR1* sequencing in the neuromuscular clinic are myalgia, muscle cramps, and muscle weakness[18,19]
7. Family history of an *RYR1* variant detected during diagnostic testing in the neuromuscular clinic for symptoms suggestive of a neuromuscular disorder but without a specific clinical or histopathologic diagnosis and/or fulfilling one of the other referral criteria
8. Personal history of idiopathically elevated resting creatine kinase values
9. Personal history of exertional heat stroke defined as a temperature higher than 40°C/104°F with central nervous system dysfunction
10. Other

Final Diagnosis after Full MH Diagnostic Process

Based on the available information concerning genetic and CHCT/*in vitro* contracture test results, all medical records were classified as MH-susceptible, non–MH-susceptible, or unknown. Those who tested positive for both halothane and caffeine, positive for halothane only, or positive for caffeine only by CHCT/*in vitro* contracture test were

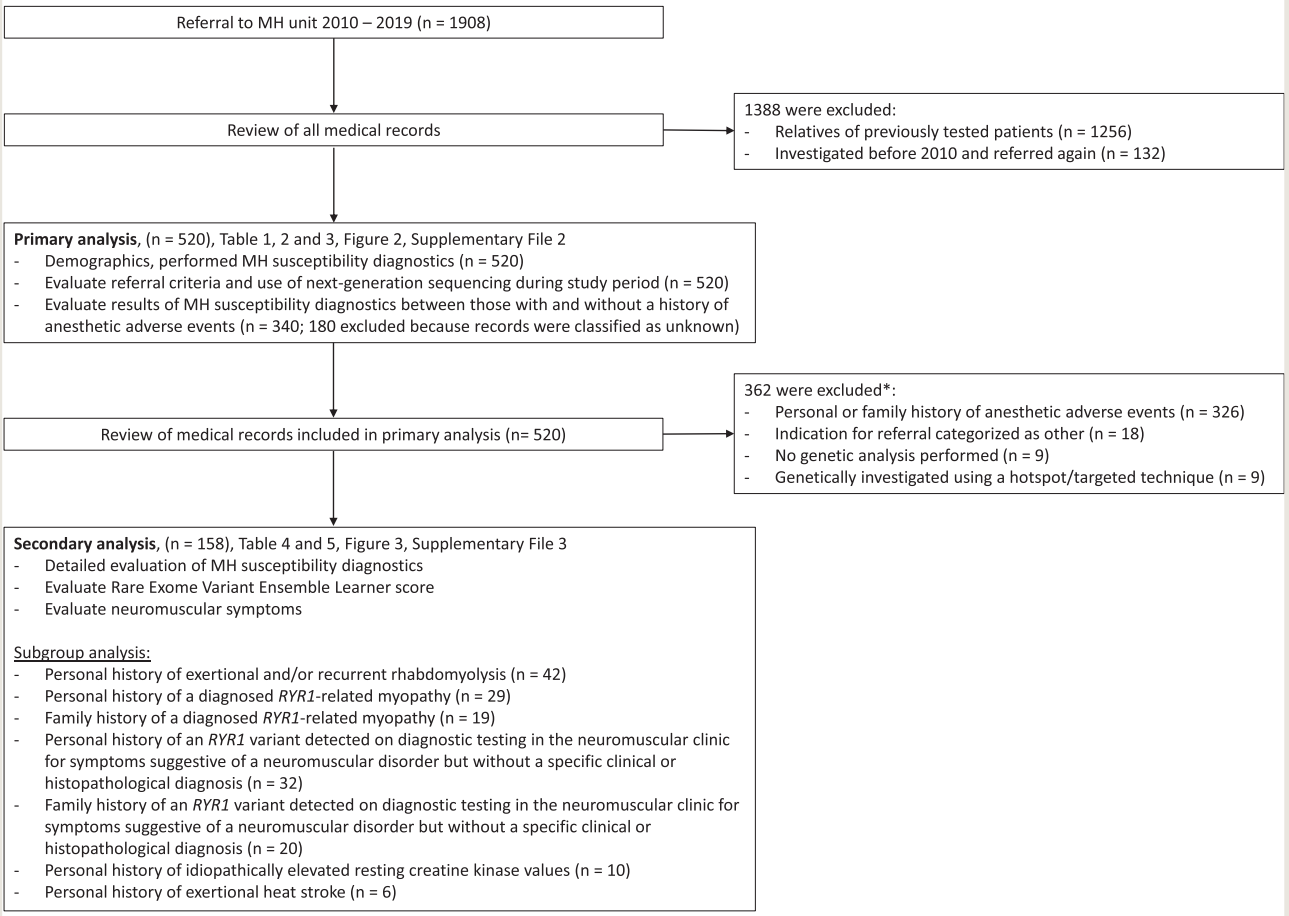

Fig. 1. A summary of the study design and study selection process. *Some medical records meet more than one exclusion criterion. MH, malignant hyperthermia.

classified as MH-susceptible. Those who were tested negative for both halothane and caffeine by CHCT/*in vitro* contracture test were classified as non–MH-susceptible. Patients with an *RYR1*/*CACNA1S* variant diagnostic for MH according to the European Malignant Hyperthermia Group list of diagnostic variants (www.emhg.org/diagnostic-mutations; Lund, Sweden) were classified as MH-susceptible.[20]

In cases in which the medical records did not support any of the above criteria, the records were classified as unknown.[4] Based on the available information concerning the diagnostic procedures performed, these records could not be classified as MH-susceptible or non–MH-susceptible because the relevant investigations (genetic testing and/or CHCT/*in vitro* contracture test) were not (yet) performed due to the waiting lists in the participating MH units, patient's refusal, missing data, or a combination of these reasons.

Individuals referred to the MH unit in Toronto were investigated by CHCT according to the North American MH protocol. The CHCT has a sensitivity of 97% and specificity of 78%.[1,2] Individuals referred to Nijmegen, Lund, and Antwerp were investigated by *in vitro* contracture test according to the European Malignant Hyperthermia Group protocol. The *in vitro* contracture test has a sensitivity of 100% and specificity of 94%.[3,4]

RYR1 Pathogenicity Classification Using Computational Evidence

To study whether computational evidence is useful when counseling patients without a history of an adverse anesthetic event suspected to be MH, the Rare Exome Variant Ensemble Learner score[21] was used. During the secondary analysis, the Rare Exome Variant Ensemble Learner score was calculated for the *RYR1* missense variants that were not on the European Malignant Hyperthermia Group

list of diagnostic variants.[20] As the Rare Exome Variant Ensemble Learner score was developed for missense variants, other types of *RYR1* variants (*e.g.*, duplication or deletion variants) were excluded from the analysis. When two or more *RYR1* missense variants were identified, the variant with the highest Rare Exome Variant Ensemble Learner score was included in the analysis. According to the ClinGen Variant Curation Expert Panel for *RYR1* pathogenicity classification recommendations for *RYR1* pathogenicity assertions in MH susceptibility,[10] a Rare Exome Variant Ensemble Learner score of 0.5 or lower is evidence against pathogenicity, a Rare Exome Variant Ensemble Learner score of 0.5 to 0.85 neither is evidence against nor supports pathogenicity, and a Rare Exome Variant Ensemble Learner score of 0.85 or higher supports pathogenicity.

Statistical Analysis

The sample size was based on the available data. Therefore, no statistical power analysis was performed. Normality of continuous variables was assessed with the use of histograms. Continuous variables were reported as mean ± SD for normally distributed data and as median and interquartile range for nonnormally distributed data. Categorical variables were reported using frequencies and percentages. The chi-square test was used to compare categorical variables. Records classified as unknown were included in the descriptive statistics regarding MH diagnostic tests used and also in the analysis regarding the referral criteria during the study period. These records were excluded from the statistical analysis comparing the proportion of records classified as MH-susceptible between those with or without personal or family history of adverse anesthetic events suspected to be MH. All statistical analyses were performed using IBM SPSS Statistics for Windows version 24.0 (USA). A two-tailed P value < 0.05 was used as a cutoff for significance. The statistical analysis plan, definition of the subgroups, and outcomes were documented before accessing the data.

Results

Primary Analysis

Demographic Characteristics. In the primary analysis, 520 medical records were included (fig. 1). Median age at referral was 37 yr (interquartile range, 28 to 50), and 265 (51.0%) were male. Based on the information in their MH unit medical records, 180 (34.6%) patients were categorized as MH-susceptible, 160 (30.8%) were categorized as non–MH-susceptible, and 180 (34.6%) were categorized as unknown. In seven (1.8%) medical records, the result of the genetic analysis could not be identified. There were no missing CHCT/*in vitro* contracture test results. The clinical grading scale[17] was available for 170 probands, of which 109 completed the full diagnostic process of MH susceptibility. Resting creatine kinase values were available in 134 (25.8%) medical records, of which 115 completed the full diagnostic process for MH susceptibility. The demographic characteristics are summarized in table 1.

Referral Indications and Use of Next-generation Sequencing during the Study Period. The most frequent referral indication was a personal history of an anesthetic adverse event suspected to be MH (n = 211; 40.6%). A total of 194 (37.3%) patients referred to the participating MH units did not have a personal or family history of adverse anesthetic events suspected to be MH. Referral indications changed during the study period; 28.4% (61 of 215) of the patients referred between 2010 to 2014 did not have a personal or family history of an adverse anesthetic event suspected to be MH, while this increased to 43.6% (133 of 305) between 2015 to 2019 (P < 0.001). Distribution of the referral criteria for each MH unit is summarized in table 2.

A CHCT/*in vitro* contracture test was performed in 288 (55.4%) patients, genetic investigation was performed in 399 (76.7%) patients, and 192 (36.9%) were investigated both genetically and by CHCT/*in vitro* contracture test. Of the 399 genetically investigated patients, 168 (42.1%) were investigated by sequencing of both the *RYR1* and *CACNA1S* genes. The use of next-generation sequencing increased during the study period; 49.3% (106 of 215) of the patients referred between 2010 to 2014 were investigated by next-generation sequencing, while this increased to 68.2% (208 of 305) between 2015 and 2019 (P < 0.001). Referral indication and the use of next-generation sequencing during the study period are summarized in figure 2.

In the subgroup of those without a personal or family history of an adverse anesthetic event suspected to be MH (n = 194), 47 were diagnosed as MH-susceptible, 73 were diagnosed as non–MH-susceptible, and 74 were classified as unknown. Patients with a personal or family history of an anesthetic adverse event suspected to be MH were more frequently diagnosed as MH-susceptible (133 of 220; 60.5%) than those without (47 of 120; 39.2%; P < 0.001). A total of 180 records were excluded from the analysis because of insufficient information, resulting in classification as unknown. Referral indications and details of the diagnostic tests performed are summarized in table 1.

The results of the genetic analysis, overall and for the subgroups (MH-susceptible, non–MH-susceptible, and unknown), are summarized in table 3. We did not identify any *STAC3* variants; however, this gene was specifically investigated in only one patient. All identified variants in the *RYR1* and *CACNA1S* genes and in other genes relevant for the neuromuscular clinic are given in Supplemental Digital Content 2 (http://links.lww.com/ALN/C834).

Table 1. Patient Characteristics, Referral Indications, and Diagnostic Tests Performed

Characteristics and Analyses	MH-susceptible, n (%)	Non–MH-susceptible, n (%)	Unknown, n (%)	Total, n
MH investigation unit				
Antwerp (Belgium)	36 (43.9)	45 (54.9)	1 (1.2)	82
Lund (Sweden)	55 (50.9)	37 (34.3)	16 (14.8)	108
Nijmegen (The Netherlands)	31 (21.1)	64 (43.5)	52 (35.4)	147
Toronto (Canada)	58 (31.7)	14 (7.7)	111 (60.7)	183
Total	180 (34.6)	160 (30.8)	180 (34.6)	520
Median age at referral, yr [interquartile range]	36 [26 to 48]	38 [31 to 50]	39 [27 to 53]	37 [28 to 50]
Sex				
Male	118 (44.5)	56 (21.1)	91 (34.3)	265
Female	61 (24.0)	104 (40.9)	89 (35.0)	254
Unknown	1 (100.0)	0 (0.0)	0 (0.0)	1
Referral indication				
Personal history of adverse anesthetic events suspected to be MH	93 (44.1)	42 (19.9)	76 (36.0)	211
Family history of adverse anesthetic events suspected to be MH	40 (34.8)	45 (39.1)	30 (26.1)	115
Exertional and recurrent rhabdomyolysis	19 (41.3)	10 (21.7)	17 (37.0)	46
Personal *RYR1* variant detected in neuromuscular clinic testing without specific diagnosis	13 (35.1)	13 (35.1)	11 (29.7)	37
A diagnosed *RYR1*-related myopathy	6 (18.8)	13 (40.6)	13 (40.6)	32
Family history of an *RYR1* variant detected in diagnostic testing without specific diagnosis	2 (10.0)	11 (55.0)	7 (35.0)	20
Family history of diagnosed *RYR1*-related myopathy	1 (5.3)	9 (47.4)	9 (47.4)	19
Idiopathically elevated resting creatine kinase values	1 (7.1)	6 (42.9)	7 (50.0)	14
Exertional heat stroke	2 (25.0)	2 (25.0)	4 (50.0)	8
Other	3 (16.7)	9 (50.0)	6 (33.3)	18
Clinical grading scale in points [interquartile range] (n = 170)	35 [23 to 44]	17 [15 to 20]	35 [30 to 43]	33 [18 to 40]
Resting creatine kinase values in U/l [interquartile range] (n = 134)	301 [156 to 708]	102 [72 to 187]	567 [217 to 854]	153 [83 to 476]
Type of diagnostic tests performed				
CHCT/*in vitro* contracture test performed	128 (44.4)	160 (55.6)	0 (0.0)	288
Genetic testing performed	155 (38.8)	88 (22.1)	156 (39.1)	399
CHCT/*in vitro* contracture test and genetic testing were both performed	104 (54.2)	88 (45.8)	0 (0.0)	192
Type of genetic tests performed				
RYR1 hot spots or familial *RYR1*/*CACNA1S*-variant	39 (60.0)	13 (20.0)	13 (20.0)	65
RYR1 + *CACNA1S* sequencing (entire genes)	53 (31.5)	13 (7.7)	102 (60.7)	168
RYR1 sequencing (entire gene)	45 (45.0)	26 (26.0)	29 (29.0)	100
Whole-exome sequencing	7 (26.9)	14 (53.8)	5 (19.2)	26
RYR1 + *CACNA1S* + *STAC3* sequencing (entire genes)	0 (0.0)	0 (0.0)	1 (100.0)	1
Other (*e.g.*, *RYR1* + other relevant genes for neuromuscular diagnostics)	4 (19.0)	13 (61.9)	4 (19.0)	21
Unknown	8 (44.4)	9 (50.0)	1 (5.6)	18

The table shows the patient characteristics, referral indications, and diagnostic tests performed for all patients referred from 2010 through 2019 (n = 520).
CHCT, caffeine–halothane contracture test; MH, malignant hyperthermia.

Table 2. Distribution of Referral Criteria for Each MH Unit

Referral Indication	Antwerp, n (%)	Lund, n (%)	Nijmegen, n (%)	Toronto, n (%)
Personal history of anesthetic adverse event suspected to be MH	19 (23.2)	77 (71.3)	42 (28.6)	73 (39.9)
Family history of anesthetic adverse event suspected to be MH	33 (40.2)	22 (20.4)	19 (12.9)	41 (22.4)
Exertional and recurrent rhabdomyolysis	7 (8.5)	2 (1.9)	12 (8.2)	25 (13.7)
RYR1 variant detected in diagnostic testing without a specific diagnosis	4 (4.9)	1 (0.9)	27 (18.4)	5 (2.7)
A diagnosed *RYR1*-related myopathy	4 (4.9)	2 (1.9)	16 (10.9)	10 (5.5)
Family history of an *RYR1* variant detected in diagnostic testing without a specific diagnosis	2 (2.4)	0 (0.0)	16 (10.9)	2 (1.1)
Family history of a diagnosed *RYR1*-related myopathy	3 (3.7)	1 (0.9)	7 (4.8)	8 (4.4)
Idiopathically elevated resting creatine kinase values	4 (4.9)	0 (0.0)	1 (0.7)	9 (4.9)
Exertional heat stroke	0 (0.0)	1 (0.9)	1 (0.7)	6 (3.3)
Other	6 (7.3)	2 (1.9)	6 (4.1)	4 (2.2)
Total	82	108	147	183

MH, malignant hyperthermia.

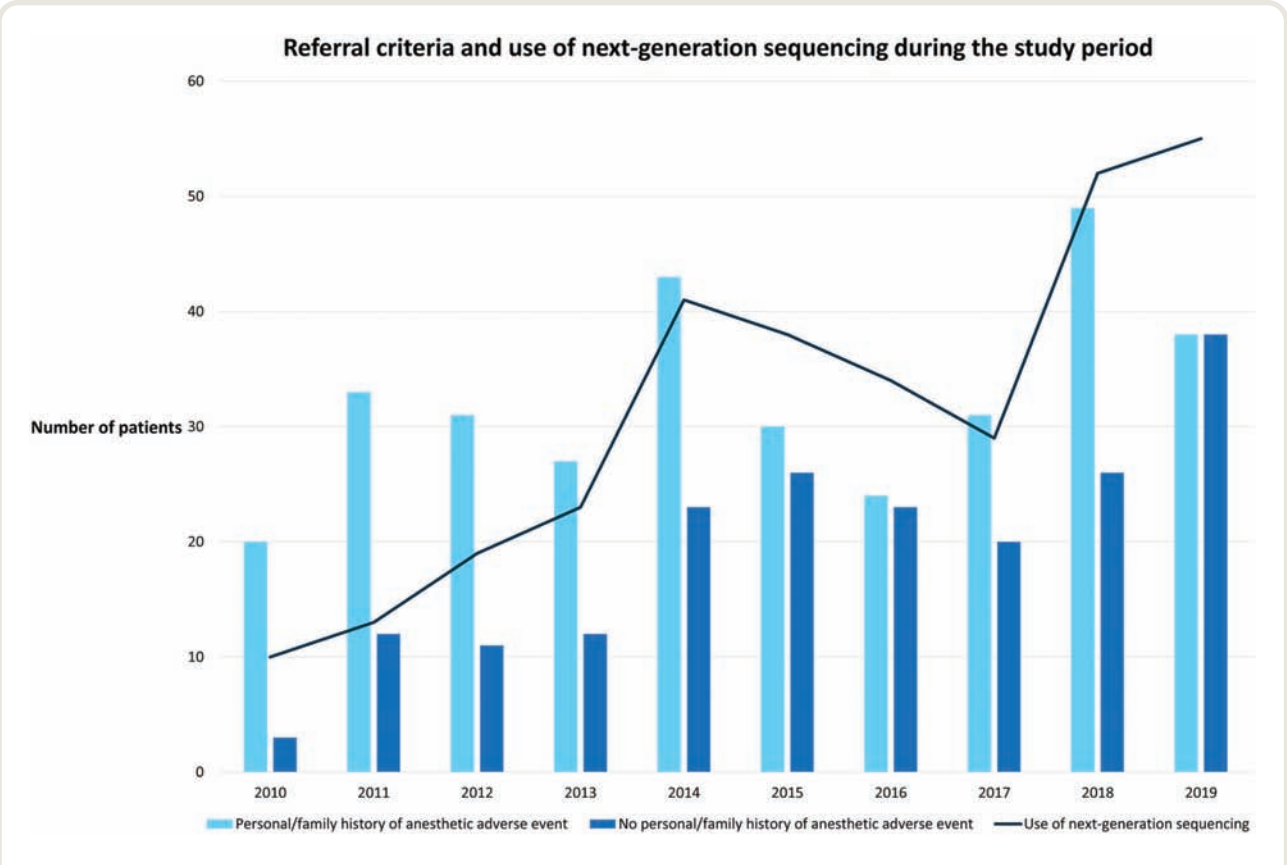

Fig. 2. Referral criteria and use of next-generation sequencing during the study period.

Secondary Analysis

Results of MH Diagnostics of Patients without a Personal or Family History of an Adverse Anesthetic Event Suspected to Be MH. The medical records of 158 patients without a personal or family history of an anesthetic adverse event suspected to be MH were included in the secondary analysis (fig. 1). A total of 42 referred because of a personal history of exertional and/or recurrent rhabdomyolysis were included in the secondary analysis. Based on the information contained in these records, 16 of 42 were diagnosed as MH-susceptible. Only one of six patients referred because of exertional heat stroke was diagnosed as MH-susceptible.

Table 3. Results of Genetic Testing of Patients Investigated by Genotyping

Test Result	MH-susceptible (n = 155), %	Non–MH-susceptible (n = 88), %	Unknown (n = 156), %	Total (n = 399), %
No variant in *RYR1* or *CACNA1S*	43 (27.7)	32 (36.4)	74 (47.4)	149 (37.3)
Variant(s) of unknown significance in *RYR1*	27 (17.4)	49 (55.7)	65 (41.7)	141 (35.3)
Diagnostic *RYR1* variant for MH susceptibility	67 (43.2)	0 (0.0)	0 (0.0)	67 (16.8)
Variant(s) of unknown significance in *CACNA1S*	5 (3.2)	1 (1.1)	9 (5.8)	15 (3.8)
RYR1 variant(s) + variant in other relevant gene(s) for neuromuscular diagnostics	2 (1.3)	5 (5.7)	3 (1.9)	10 (2.5)
Diagnostic *CACNA1S* variant for MH susceptibility	2 (1.3)	0 (0.0)	0 (0.0)	2 (0.5)
Diagnostic *RYR1/CACNA1S* variant for MH susceptibility + variant of unknown significance in *RYR1/CACNA1S*	4 (2.6)	0 (0.0)	0 (0.0)	4 (1.0)
Variant of unknown significance in *RYR1* + *CACNA1S*	1 (0.6)	0 (0.0)	3 (1.9)	4 (1.0)
Unknown	4 (2.6)	1 (1.1)	2 (1.3)	7 (1.8)

Percentages of the performed tests are given for all patients included in the study and per subgroup (MH-susceptible, non–MH-susceptible, and unknown). In the medical records of seven patients, the results of the genetic testing could not be identified.

MH, malignant hyperthermia.

However, only two of six were investigated by CHCT/*in vitro* contracture test. The details of the CHCT/*in vitro* contracture test and genetic analysis results from patients referred because of a personal history of exertional and/or recurrent rhabdomyolysis and exertional heat stroke are summarized in table 4.

Of 29 patients referred for a *RYR1*-related myopathy, 5 were diagnosed MH-susceptible. Of 19 patients for whom referral was for a family history of *RYR1*-related myopathy, 1 was diagnosed as MH-susceptible. A total of 11 of 32 patients were diagnosed as MH-susceptible when the referral indication was an *RYR1* variant detected in patients seen in the neuromuscular clinic and tested for symptoms suggestive of a neuromuscular disorder but without a specific clinical or histopathologic diagnosis. Of 20 patients for whom a family history of an *RYR1* variant detected in diagnostic testing in the neuromuscular clinic without a specific clinical or histopathologic diagnosis was the referral indication in the medical records, 2 were diagnosed as MH-susceptible. Idiopathically elevated creatine kinase level was the referral indication in the medical records of 10 patients, where 1 was diagnosed as MH-susceptible.

The results of the performed CHCT/*in vitro* contracture test and genetic analysis of patients referred because of a personal or family history of *RYR1*-related myopathies, idiopathically elevated creatine kinase values, and a personal or family history of an *RYR1* variant detected on diagnostic testing in the neuromuscular clinic are summarized in table 5. All neuromuscular symptoms reported in the medical records of patients with an *RYR1* variant detected in the neuromuscular clinic without a specific clinical or histopathologic diagnosis are summarized in Supplemental Digital Content 3 (http://links.lww.com/ALN/C835).

RYR1 *Pathogenicity Classification Using Computational Evidence.* A total of 71 patients without a personal or family of an adverse anesthetic event suspected to be MH had at

Table 4. Results of Genetic Analysis and CHCT/*In Vitro* Contracture Test of Patients with Episodic *RYR1*-related Phenotypes

Characteristics, Analyses, and Test Results	Exertional and/or Recurrent Rhabdomyolysis (n = 42)	Exertional Heat Stroke (n = 6)
Sex		
Male	32	4
Female	10	2
Genes analyzed		
RYR1 + *CACNA1S*	25	5
RYR1	8	1
RYR1 + other relevant genes for neuromuscular diagnostics	8	0
Whole-exome sequencing	1	0
Results of genetic analysis and CHCT/*in vitro* contracture test categorized according to the result of the genetic analysis		
Diagnostic *RYR1* variant for MH (total)	6	0
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0
No CHCT/*in vitro* contracture test performed	5	0
Variant of unknown significance in *RYR1* (total)	16	1
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	2	0
Tested positive for halothane only by CHCT/*in vitro* contracture test	1	0
Non–MH-susceptible	6	1
No CHCT/*in vitro* contracture test performed	7	0
Diagnostic *RYR1* variant for MH susceptibility + variant of unknown significance in *RYR1* (total)	1	0
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0
Diagnostic *CACNA1S* variant for MH (total)	1	1
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	1
Variant of unknown significance in *CACNA1S* (total)	5	1
Tested positive for halothane only by CHCT/*in vitro* contracture test	2	0
No CHCT/*in vitro* contracture test performed	3	1
No variant in *RYR1*/*CACNA1S* (total)	12	2
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0
Tested positive for halothane only by CHCT/*in vitro* contracture test	1	0
Tested positive for caffeine only by CHCT/*in vitro* contracture test	1	0
Non–MH-susceptible	3	0
No CHCT/*in vitro* contracture test performed	6	2
RYR1 variant + variant(s) in other relevant gene(s) (total)*	1	1
Non–MH-susceptible	1	0
No CHCT/*in vitro* contracture test performed	0	1

Shown are the results of the genetic analysis and the CHCT/*in vitro* contracture test of 48 patients referred because of the episodic *RYR1*-related phenotypes (exertional and/or recurrent rhabdomyolysis and exertional heat stroke).

*Genes relevant for the neuromuscular clinic.

CHCT, caffeine–halothane contracture test; MH, malignant hyperthermia.

least one missense *RYR1* variant. Most of the Rare Exome Variant Ensemble Learner scores (37 of 71) were between 0.5 and 0.85 and therefore not helpful in *RYR1* pathogenicity classification; 9 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores of 0.5 or lower, and 25 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores of 0.85 or higher.

A total of 34 of 71 *RYR1* variants had a Rare Exome Variant Ensemble Learner score of 0.5 or lower or 0.85 or higher, indicating a benign (0.5 or lower) or pathogenic

Table 5. Results of Genetic Analysis and CHCT/*In Vitro* Contracture Test of Patients with History of *RYR1*-related Myopathies, Idiopathically Elevated Creatine Kinase Values, and History of *RYR1* Variant

Characteristics and Analyses	A Diagnosed *RYR1*-related Myopathy (n = 29)	Family History of Diagnosed *RYR1*-related Myopathy (n = 19)	*RYR1* Variant Detected in Neuromuscular Diagnostics (n = 32)	Family History of *RYR1* Variant Detected in Neuromuscular Diagnostics (n = 20)	Idiopathically Elevated Resting Creatine Kinase Values (n = 10)
Sex					
Male	12	8	21	6	4
Female	17	11	11	14	6
Genes analyzed					
RYR1 + *CACNA1S*	8	8	5	2	8
RYR1	12	3	10	5	0
RYR1 targeted/hotspot technique	Not applicable	5	Not applicable	12	Not applicable
RYR1 + other relevant genes	2	0	8	1	1
Whole-exome sequencing	7	3	9	0	0
RYR1 + *CACNA1S* + *STAC3*	0	0	0	0	1
Results genetic analysis and CHCT/*in vitro* contracture test categorized according to results of the genetic analysis					
Diagnostic *RYR1* variant for MH (total)	2	1	5	0	1
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	0	0	2	0	1
Tested positive for halothane only by CHCT/*in vitro* contracture test	0	0	0	0	0
Tested positive for caffeine only by CHCT/*in vitro* contracture test	0	0	1	0	0
No CHCT/*in vitro* contracture test performed	2	1	2	0	0
Variant of unknown significance in *RYR1* (total)	19	13	20	18	2
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0	3	1	0
Tested positive for halothane only by CHCT/*in vitro* contracture test	0	0	0	1	0
Tested positive for caffeine only by CHCT/*in vitro* contracture test	1	0	1	0	0
Non–MH-susceptible	9	4	9	3	2
No CHCT/*in vitro* contracture test performed	8	9	7	13	0
Diagnostic *RYR1* variant for MH + variant of unknown significance in *RYR1* (total)	0	0	1	0	0
No CHCT/*in vitro* contracture test performed	0	0	1	0	0
Variant of unknown significance in *CACNA1S* (total)	0	0	0	0	1
No CHCT/*in vitro* contracture test performed	0	0	0	0	1
No variant in *RYR1*/*CACNA1S*/STAC3 (total)	6	3	0	1	6
Non–MH-susceptible	1	2	0	0	1
No CHCT/*in vitro* contracture test performed	5	1	0	1	5
RYR1 variant(s) + variant in other relevant gene(s)* (total)	2	1	6	1	0
Tested positive for both halothane and caffeine by CHCT/*in vitro* contracture test	1	0	1	0	0
Non–MH-susceptible	1	0	2	1	0
No CHCT/*in vitro* contracture test performed	0	1	3	0	0
Results of genetic analysis unknown	0	1	0	0	0

Shown are the results of the genetic analysis and the CHCT/*in vitro* contracture test of 110 patients referred because of a personal or family history of *RYR1*-related myopathies, idiopathically elevated creatine kinase values, and a personal or family history of an *RYR1* variant detected on diagnostic testing in the neuromuscular clinic. The patients with the following myopathies were classified as *RYR1*-related myopathies: axial myopathy, central core disease, King–Denborough syndrome, fiber disproportion disorder, periodic paralysis, centronuclear myopathy, and multiminicore disease.

*Genes relevant for the neuromuscular clinic.

CHCT, caffeine–halothane contracture test; MH, malignant hyperthermia.

(0.85 or higher) variant. The full diagnostic process of MH susceptibility was completed by 19 of 34. In 10 of 19, the Rare Exome Variant Ensemble Learner scores were discordant with the results of MH susceptibility diagnostics. Rare Exome Variant Ensemble Learner scores and the results of MH susceptibility diagnostics are summarized in figure 3.

Discussion

This retrospective multicenter cohort study shows that the indications for referral to MH units have changed. An increasing number of patients referred to MH units do not have a personal or family history of an adverse anesthetic event suspected to be MH. This trend coincides with the publication of the European Malignant Hyperthermia Group guideline for investigation of MH susceptibility in 2015.[4] This guideline recommends referral to an MH unit for patients with exertional and/or recurrent rhabdomyolysis, *RYR1*-related myopathies, and other *RYR1*-related phenotypes. This might, at least partly, explain the increasing number of referrals concerning patients without a personal or family history of an adverse anesthetic event. These patients carry *RYR1* variants identified during the diagnostic workup for exertional and/or recurrent rhabdomyolysis, exertional heat stroke, *RYR1*-related myopathies, or an unresolved nonspecific neuromuscular phenotype reflecting the wide spectrum of *RYR1*-related phenotypes.[8] Since 39.2% of the patients referred to an MH unit without a personal or family history of an anesthetic adverse event suspected to be MH were diagnosed as MH-susceptible, these patients can be at risk of MH when exposed to triggering anesthetic agents. On the other hand, 60.8% of the patients without a personal or family history of an adverse anesthetic event were diagnosed as non–MH-susceptible, indicating the importance of MH susceptibility diagnostics; a non–MH-susceptible test result enables anesthesiologists to treat carriers of *RYR1* variants and their family members without MH precaution measures.[4,22]

In our study, 16 of 42 of the patients with exertional and/or recurrent rhabdomyolysis were diagnosed as MH-susceptible, which compared to previous case studies is less frequent than 11 of 12[23] and 5 of 6[24] but more frequent than 2 of 14.[18] This variability can, at least partly, be explained by selection bias concerning some of these study cohorts. Another cohort study reporting 17 MH-susceptible patients who suffered more than two episodes of exertional rhabdomyolysis identified 9 patients with *RYR1* variants, including two pathogenic variants for MH.[11] Previous studies on MH susceptibility in exertional heat stroke patients reported a positive *in vitro* contracture test in 12 of 28,[25] which is higher than in our study, probably due to the low sample size of the exertional heat

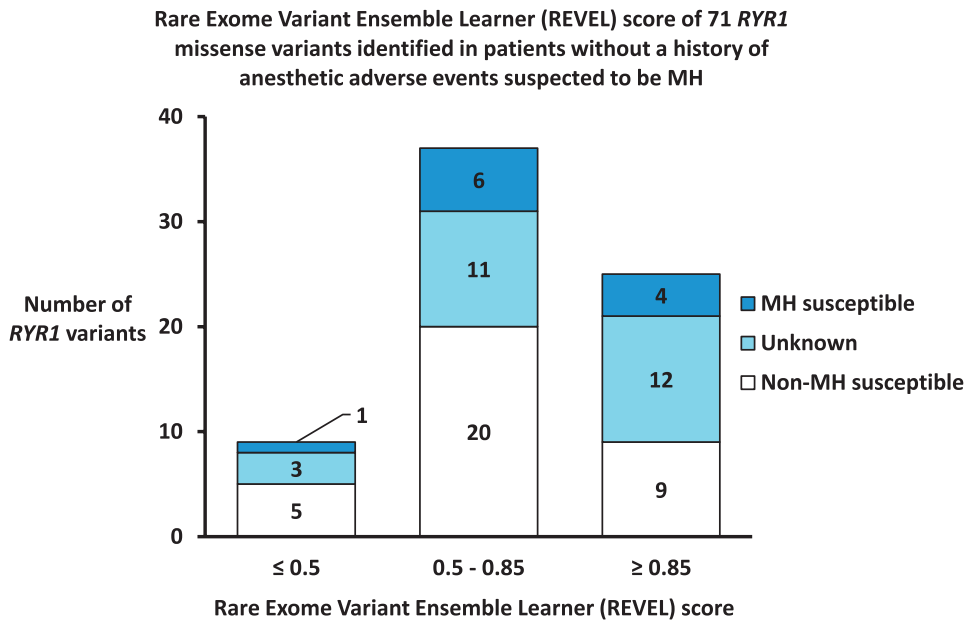

Fig. 3. Rare Exome Variant Ensemble Learner cores of 71 *RYR1* missense variants identified in patients without a history of anesthetic adverse events had a missense variant of unknown significance in *RYR1*. A total of 37 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores between 0.5 and 0.85 and therefore were not helpful in *RYR1* pathogenicity classification. A total of 9 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores of 0.5 or lower, of which 1 record was classified MH-susceptible. A total of 25 of 71 *RYR1* variants had Rare Exome Variant Ensemble Learner scores of 0.85 of higher, of which 9 records were classified as non–MH-susceptible. MH, malignant hyperthermia.

stroke cohort and the high number of patients classified as unknown in our study.

There are several case reports reporting MH reactions and studies reporting MH susceptibility in patients with *RYR1*-related myopathies,[26–29] but we did not identify any cohort studies or large case series on MH susceptibility in patients with *RYR1*-related myopathies. The same applies to patients with an *RYR1* variant detected in diagnostic testing in the neuromuscular clinic without a specific clinical or histopathologic diagnosis.[18]

As next-generation sequencing has become faster and more cost-effective, it is gradually becoming the first-line diagnostic test for genetically heterogenous disorders (such as congenital myopathies). This has resulted in a rapid increase in the identification of both the number of patients with an *RYR1* variant and the number of newly identified *RYR1* variants. Only a limited number of these *RYR1* variants are classified as pathogenic or benign according to the Variant Curation Expert Panel recommendations for *RYR1* pathogenicity classification in MH susceptibility[10] and/or the European Malignant Hyperthermia Group scoring matrix for classification of genetic variants in MH susceptibility. However, only variants classified as (likely) pathogenic or benign can be used for genetic MH susceptibility diagnostics.[10] The increasing number of patients without a personal or family history of an adverse anesthetic event with a variant of unknown significance in *RYR1* will be a major challenge for MH units in future.

As our study shows, bioinformatic prediction tools are currently insufficient to classify *RYR1* missense variants of unknown significance. In 37 of 71 of the patients with a missense variant of unknown significance in *RYR1* who did not have a history of an adverse anesthetic event, the Rare Exome Variant Ensemble Learner scores were between 0.5 and 0.85 and were therefore not helpful.[10] Furthermore, the Rare Exome Variant Ensemble Learner score does not take into consideration the possibility of two or more *RYR1* variants interacting in a synergistic manner with regards to their pathogenicity. Rare Exome Variant Ensemble Learner scores of 0.5 or lower and 0.85 or higher may be useful as preliminary guidance but are currently not validated to confirm or rule out MH susceptibility. We identified 10 of 19 cases of discordance between the CHCT/*in vitro* contracture test result and the Rare Exome Variant Ensemble Learner score (fig. 3). These findings are in line with other *in silico* predictors of pathogenicity in MH.[30,31]

Our results can be used to improve counseling of patients referred to MH units without a personal or family history of an adverse anesthetic event suspected to be MH. Furthermore, these results can also be useful for geneticists, neurologists, and other specialists investigating patients by *RYR1* sequencing or whole-exome sequencing. They need to be aware of and inform patients about the possibility of identifying a variant of unknown significance in *RYR1* and the potential subsequent need for a muscle biopsy for CHCT/*in vitro* contracture test and, if relevant, cascade family testing in all first-degree family members before they perform *RYR1* sequencing (either targeted or by next-generation sequencing).

Our study has some limitations. Some referral indications were disproportionally distributed between the participating MH units, probably caused by the close collaboration between the MH units in Toronto and Nijmegen and the university hospital neuromuscular clinic, in contrast to the MH unit in Lund, which does not have any collaborations with the local neurology department. In addition, only a limited number of patients were tested for *CACNA1S* and *STAC3* variants, and several patients referred because of a family history of an *RYR1*-related myopathy (5 of 19) or a family history of an *RYR1* variant detected in diagnostic testing in the neuromuscular clinic without a specific clinical or histopathologic diagnosis (12 of 20) were genetically investigated using a targeted technique. Therefore, we are not sure whether these unaffected family members carried *RYR1* variant(s) other than those identified in their relatives with neuromuscular symptoms. Furthermore, due to the low penetrance of MH susceptibility,[10,12,32] currently unresolved modifying factors in the occurrence of MH, and ethical limitations, it is not possible to study which MH-susceptible patients suffer an MH reaction when exposed to triggering agents. Since the CHCT/*in vitro* contracture test and screening for diagnostic variants are the accepted standard in MH susceptibility diagnostics[4] and an MH reaction can be life-threatening,[13,33] all patients diagnosed as MH-susceptible should be considered at risk for MH when in need of anesthesia.[22]

It is important to mention that not all patients who suffer a rhabdomyolysis and/or exertional heat stroke episode will be referred to MH units as only a limited number of patients have a genetic background associated with an increased susceptibility to rhabdomyolysis[34] or exertional heat stroke.[25,35,36] Neurologists and sport physicians only refer patients to an MH unit with signs of an increased genetic susceptibility to rhabdomyolysis and/or exertional heat stroke, resulting in a selection bias. The same selection bias arises for the *RYR1* variants within the study cohort. Patients with *RYR1* variants resulting in a loss of function in ryanodine receptor 1 or a high prevalence in control populations are unlikely to cause MH.[10] Carriers of these variants are therefore less likely to be referred to an MH unit.

Last, the number of patients in our cohort who did not complete the full process of MH susceptibility diagnostics might have affected our results but probably also reflect the problem our study addresses. The CHCT/*in vitro* contracture test is an invasive procedure, and some patients referred for MH susceptibility diagnostics refuse muscle biopsy or are unable to undergo muscle biopsy and consider themselves MH-susceptible without confirmation of the diagnosis. Furthermore, worldwide knowledge and expertise needed to perform a reliable CHCT/*in vitro* contracture test are limited.

In some countries, there are no CHCT/*in vitro* contracture test laboratories, and the established MH units in other countries have long waiting lists, resulting in a very large number of patients carrying *RYR1* variants of unknown significance with limited possibilities for MH susceptibility diagnostics. This is also the case for the MH unit in Toronto; most patients who did not complete the full process of MH susceptibility are on the waiting list to be investigated.

Future strategies for MH susceptibility diagnostics should focus on classification of *RYR1*, *CACNA1S*, and *STAC3* variants utilizing common databases and functional studies. This should not be limited to suspected pathogenic variants because classification of a variant as benign could prevent unnecessary invasive diagnostic procedures. Other potential fields of interest for future research are identification of new genes of interest as 27.7% of the MH-susceptible–diagnosed patients did not have a variant in *RYR1*, *CACNA1S*, or *STAC3*. As our study demonstrates, currently available bioinformatic models such as Rare Exome Variant Ensemble Learner[21] are insufficient for MH susceptibility diagnostics, but more useful alternatives may emerge in the future.

Conclusions

The proportion of patients referred to MH units without a personal or family history of adverse anesthetic events suspected to be MH has increased. These patients carry *RYR1* variants identified during the diagnostics workup for exertional or recurrent rhabdomyolysis, exertional heat stroke, *RYR1*-related myopathies, or an unresolved neuromuscular phenotype. Since 39.2% of the patients referred to an MH unit without a personal or family history of an anesthetic adverse event suspected to be MH were diagnosed as MH-susceptible, these patients can be at risk for MH when exposed to MH triggering agents, and the referral of such patients to MH units is therefore indicated.

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Competing Interests

Dr. Heytens is supported by Norgine BV (Amsterdam, The Netherlands). The other authors declare no competing interests.

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ANESTHESIOLOGY

Midazolam at Low Nanomolar Concentrations Affects Long-term Potentiation and Synaptic Transmission Predominantly *via* the α_1–γ-Aminobutyric Acid Type A Receptor Subunit in Mice

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Administration of the benzodiazepine midazolam induces anterograde amnesia *via* γ-aminobutyric acid type A ($GABA_A$) receptor–dependent mechanisms
- Midazolam blocks hippocampal long-term potentiation, a cellular correlate for learning and memory
- The specific $GABA_A$ receptor subunits mediating the amnestic actions of midazolam are incompletely understood

What This Article Tells Us That Is New

- Using a combination of γ-aminobutyric acid type A ($GABA_A$) α-receptor subunit knock-in mice revealed that low concentrations (10 nM) of midazolam blocked long-term potentiation in the hippocampal slice preparation predominantly *via* α_1-$GABA_A$ receptors
- Electrophysiologic recordings in neocortical slice cultures imply a dominant role for the α_1 subtype in governing inhibitory postsynaptic current kinetics at nanomolar concentrations of midazolam
- These observations suggest that, at low concentrations, midazolam enhances synaptic transmission of $GABA_A$ receptors *via* targeting α_1 subtypes and provides mechanistic explanation for the drug's sedative and amnestic action

ABSTRACT

Background: Midazolam amplifies synaptic inhibition *via* different γ-aminobutyric acid type A ($GABA_A$) receptor subtypes defined by the presence of α_1-, α_2-, α_3-, or α_5-subunits in the channel complex. Midazolam blocks long-term potentiation and produces postoperative amnesia. The aims of this study were to identify the $GABA_A$ receptor subtypes targeted by midazolam responsible for affecting CA1 long-term potentiation and synaptic inhibition in neocortical neurons.

Methods: The effects of midazolam on hippocampal CA1 long-term potentiation were studied in acutely prepared brain slices of male and female mice. Positive allosteric modulation on $GABA_A$ receptor–mediated miniature inhibitory postsynaptic currents was investigated in organotypic slice cultures of the mouse neocortex. In both experiments, wild-type mice and $GABA_A$ receptor knock-in mouse lines were compared in which α_1-, α_5-, $\alpha_{1/2/3}$-, $\alpha_{1/3/5}$- and $\alpha_{2/3/5}$-$GABA_A$ receptor subtypes had been rendered benzodiazepine-insensitive.

Results: Midazolam (10 nM) completely blocked long-term potentiation (mean ± SD, midazolam, 98 ± 11%, n = 14/8 slices/mice *vs.* control 156 ± 19%, n = 20/12; $P < 0.001$). Experiments in slices of α_1-, α_5-, $\alpha_{1/2/3}$-, $\alpha_{1/3/5}$-, and $\alpha_{2/3/5}$–knock-in mice revealed a dominant role for the α_1-$GABA_A$ receptor subtype in the long-term potentiation suppressing effect. In slices from wild-type mice, midazolam increased (mean ± SD) charge transfer of miniature synaptic events concentration-dependently (50 nM: 172 ± 71% [n = 10/6] *vs.* 500 nM: 236 ± 54% [n = 6/6]; $P = 0.041$). In $\alpha_{2/3/5}$–knock-in mice, charge transfer of miniature synaptic events did not further enhance when applying 500 nM midazolam (50 nM: 171 ± 62% [n = 8/6] *vs.* 500 nM: 175 ± 62% [n = 6/6]; $P = 0.454$), indicating two different binding affinities for midazolam to $\alpha_{2/3/5}$- and α_1-subunits.

Conclusions: These results demonstrate a predominant role of α_1-$GABA_A$ receptors in the actions of midazolam at low nanomolar concentrations. At higher concentrations, midazolam also enhances other $GABA_A$ receptor subtypes. α_1-$GABA_A$ receptors may already contribute at sedative doses to the phenomenon of postoperative amnesia that has been reported after midazolam administration.

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Midazolam is a commonly used benzodiazepine in perioperative anesthesia, causing amnesia, sedation, hypnosis, and anxiolysis. The molecular targets contributing to these actions are still unknown. Benzodiazepines such as diazepam and midazolam bind to γ-aminobutyric acid type A ($GABA_A$) receptors containing the α_1-, α_2-, α_3-. or α_5-subunits with high affinity.[1] Furthermore, a combination of pharmacologic and genetic approaches has revealed that α_1-subunit–containing $GABA_A$ receptors mediate the sedative and addictive effects, $\alpha_{2/3}$-subunit–containing receptors mediate the anxiolytic and muscle-relaxant effects,

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and α_5-subunit–containing receptors mediate at least some memory-impairing effects of benzodiazepines[2,3] and involve the depression of learning and memory-related processes in the hippocampus.[4–7] Moreover, glutamatergic cortical neurons are important players in benzodiazepine-induced sedation.[8]

Interestingly, midazolam's pharmacodynamic properties may differ from those of other benzodiazepines. While potencies for midazolam and diazepam are similar, midazolam shows a higher efficacy than diazepam at the $\alpha_1\beta_2\gamma_2$-$GABA_A$ receptors but not at the $\alpha_2\beta_2\gamma_2$-$GABA_A$ receptors.[9]

Like other benzodiazepines, midazolam produces strong anterograde amnesia.[10,11] While eliminating adverse experiences is a clinically desired effect, midazolam is also involved in producing undesired postoperative cognitive deficits.[12] Consistent with those amnesic properties, midazolam blocks hippocampal long-term potentiation, a cellular correlate for learning and memory.[5,13] A dominant role in controlling this type of synaptic plasticity has been attributed to extrasynaptically located, α_5-subunit–containing $GABA_A$ receptors generating a tonic inhibitory con ductance in CA1 pyramidal neurons.[14–17] Whereas these studies have been conducted predominantly using etomidate, midazolam's effect on long-term potentiation and the specific $GABA_A$ receptor subunit(s) in the hippocampus mediating its amnestic effects are largely unknown.

Midazolam is also highly potent in causing sedation. Studies on single– and triple–knock-in mice provided evidence that benzodiazepine-induced sedation is predominantly mediated by cortical α_1-$GABA_A$ receptors.[8,9,18] Additionally, we have shown in $\alpha_{2/3/5}$–knock-in mice that selective modulation of α_1-$GABA_A$ receptors significantly reduces high-frequency neocortical activity in the low and high γ-range.[19] However, the corresponding effects of benzodiazepines on γ-aminobutyric acid–mediated (GABAergic) synaptic transmission have not been studied in this genotype so far. Here, we have characterized the effects of midazolam on $GABA_A$ receptor–mediated synaptic currents in slices derived from wild-type and $\alpha_{2/3/5}$–knock-in mice. To identify the role of different $GABA_A$ receptor subtypes in mediating the cellular correlates for amnesia of midazolam *in vitro*, the current study is using brain slices derived from mouse lines carrying knock-in mutations in the benzodiazepine binding sites of different $GABA_A$ receptor α-subunits. It has been shown that mutations of a histidine residue into an arginine dramatically reduce benzodiazepine binding without attenuating receptor activation by the natural γ-aminobutyric acid (GABA) agonist.[20] Interestingly, even though midazolam potentiates α_5-containing $GABA_A$ receptors in a similar concentration range as α_1- and α_2-subunits, it is most efficacious on α_1.[21] This may imply that a complex interplay of all three subunits in the hippocampus is involved in the drug's amnesic properties. In the current study, we first investigated the contribution of α_1-, α_2-, and α_5-subunits on midazolam's actions on long-term potentiation evoked in hippocampal brain slices and finally detailed the midazolam effect on synaptic inhibition in wild-type and $\alpha_{2/3/5}$–knock-in mice.

Materials and Methods

Animals

All procedures were approved by the animal care committee (either Eberhard-Karls-University, Tübingen, Germany, or Technical University Munich, Munich, Germany) and were conducted in accordance with German law on animal experimentation. All efforts were made to minimize animal suffering and the number of animals used. Up to six mice were housed in a cage with *ad libitum* intake of food and water in an environmentally controlled room (23 ± 0.5°C) with a 12-h light/12-h dark cycle.

For long-term potentiation experiments, 6- to 10-week-old mice of either sex were used. Due to limited availability, older mice (17 to 19 weeks old) were used for the $\alpha_{1/3/5}$–knock-in and $\alpha_{2/3/5}$–knock-in lines. The wild-type (C57BL/6) mice were obtained from Charles River (Italy), and the α_1–knock-in (line designation: 129X1.129P2Gabra1<tm1.1Uru/Uru>10Gabra1SvRR), α_5–knock-in (line designation: 129X1-Gabra5<tm1.1Uru/Uru>), and $\alpha_{1/2/3}$–knock-in (line designation: 129X1.129P2/129P2/129T2-Gabra1<tm1.1Uru>Gabra2<tm1.1Uru>Gabra3<tm1.1Uru>GABAa123SvJ) mice were from Charles River. Genotyping from these global knock-in lines was performed by Charles River. The $\alpha_{1/3/5}$–knock-in (line designation:129X-1.129P2/129T2/129X1-Gabra1<tm1.1Uru>Gabra3<tm1.1Uru>Gabra5<t1.1-Uru>) and $\alpha_{2/3/5}$–knock-in (linedesignation:129X1.129P2/129T2/129X1>Gabra2<tm1.1Uru>Gabra3<tm1.1Uru>Gabra5<tm1.1Uru>) lines were obtained from H.-U. Zeilhofer's group at the University of Zurich (Zurich, Switzerland). The α_1–knock-in mice carry an H101R point mutation in the α_1-subunit of the $GABA_A$ receptor, and the α_5–knock-in mice carry an H105R knock-in mutation in the α_5-subunit of the $GABA_A$ receptor. Receptors containing the H-to-R mutations are fully functional, but benzodiazepines are not able to bind to the

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benzodiazepine-binding site formed in part by the α_1- and α_5-subunits of these receptors. The mutation does not alter the physiologic function of the receptor (the natural GABA ligand can still bind) but makes them resistant to modulation by allosteric modulators acting at the benzodiazepine-binding site (*e.g.*, midazolam).[9] In the triple transgenic $\alpha_{1/2/3}$–knock-in mouse line with the mutations α_1(H101R), α_2(H101R), and α_3(H126R), benzodiazepines cannot bind to α_1-, α_2-, or α_3-subunits; It binds only to α_5-subunit. Similarly, in $\alpha_{1/3/5}$–knock-in and $\alpha_{2/3/5}$–knock-in, the only available α-subunits of the $GABA_A$ receptor to which benzodiazepines can bind to are α_2 and α_1. For preparing cultured neocortical tissue slices, we used 2- to 5-day-old wild-type and $\alpha_{2/3/5}$–knock-in mice of both sexes, obtained from the sources mentioned above.

Brain Slice Preparation for Hippocampal Recordings

The mice were deeply anaesthetized with isoflurane before decapitation. The brain was rapidly removed from the head and was immediately placed in ice-cold lactated Ringer's solution containing 125 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 6 mM $MgCl_2$, 25 mM D-glucose, and 1.2 mM NaH_2PO_4 with a final pH of 7.3 and saturated with carbogen gas (95% O_2/5% CO_2). Sagittal hippocampal slices 350 μm thick were obtained using a microtome (HM 650 V; Microm International, Germany) at 4°C after cutting the whole brain into two hemispheres with a razor blade. Slices were allowed to recover at 34°C for 30 min in a chamber submerged with artificial cerebrospinal fluid containing 125 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM D-glucose, and 1.2 mM NaH_2PO_4, which was also bubbled with carbogen, and then for 1 h at room temperature before they were transferred to the recording chamber. A platinum ring with two nylon filaments was used to fix the slices in the recording chamber while carbonated fluid was continuously perfused at a flow rate of 5 ml/min. All experiments were performed at room temperature (20 to 21°C).

Brain Slice Preparation for Recording Synaptic Currents in Neocortical Neurons

Organotypic neocortical slice cultures were utilized for evaluating midazolam's effects on $GABA_A$ receptor–mediated synaptic currents. This preparation was used because neuronal population activity in this test system is sensitive to brain concentrations of midazolam *in vivo*, producing sedation and hypnosis in humans[22] (Supplemental Digital Content 1, table S1, http://links.lww.com/ALN/C845). Moreover, the study of Zeller *et al.*[8] provided evidence that cortical glutamatergic neurons mediate the motor sedative actions of benzodiazepines in mice. Slice cultures were prepared from mouse pups housed together with their mothers in a breeding facility. The same numbers of male and female pups were separated from their mothers and transferred to our laboratory. The animals were rapidly anaesthetized with isoflurane and decapitated. In accordance with local regulations of animal experimentation, extensive efforts were undertaken to minimize the time window the pups stayed without their mother. The brains were immediately isolated, cooled down in ice-cold dissection medium composed of Grey's balanced salt solution (Sigma-Aldrich, Germany) with 60 mM glucose (50%, Sigma-Aldrich) and 11 mM $MgCl_2$ (AppliChem, Germany), and fixed in the slicing chamber of a vibratome (NVSLMI motorized advance vibroslice, World Precision Instruments, Germany). Up to four brains of both sexes were mounted in the same chamber in a row. The dissection medium was added into the chamber until the brains were fully submerged. Tissue slices (thickness, 300 μm) were cut from all mounted brains simultaneously. After being separated, both female and male slices freely floated in the dissection medium, and the sex of individual slices was lost. From these tissue slices, the cortical hemispheres were manually excised with a scalpel, and each was divided into two or three slices. The neocortical slices were placed on a glass coverslip and fixed with a coagulate of chicken plasma and thrombin. The coverslips were transferred into plastic tubes containing 750 μl nutrient medium consisting of horse serum (25%), Hanks' balanced salt solution (25%), and basal medium Eagle (50%), supplemented with glutamine and glucose. The organotypic tissue cultures were maintained using the roller tube technique.[23] One day after the preparation, the medium was exchanged, and antimycotics (10 μM 5-fluoro-2-deoxyuridine, 10 μM cytosine-1β-D-arabinofuranoside, 10 μM uridine) and neuronal growth factor (10 nM) were added to reduce glial proliferation. After preparation and after each medium renewal (two times per week), the cultures were incubated 1 to 2 h in an atmosphere of 5% CO_2 under room air, attaining a pH of 7.2 to 7.4. All chemicals were obtained from Sigma-Aldrich (Germany) except the horse serum, which was obtained from Invitrogen (Germany). Electrophysiologic recordings were carried out between days 14 and 45 *ex vivo*.

Long-term Potentiation Measurements

Extracellular field excitatory postsynaptic potentials were recorded in the hippocampal CA1 stratum radiatum, evoked by stimulation in the Schaffer collateral commissural pathway of the same region using borosilicate glass micropipettes (Hugo Sachs Elektronik-Harvard Apparatus, Germany) with an open tip resistance of 1 to 2 MΩ and filled with artificial cerebrospinal fluid. Potentials were evoked by alternately delivering a test stimulus (50 μs, 5 to 20 V) through one of two bipolar tungsten electrodes (Hugo Sachs Elektronik-Harvard Apparatus, insulated to the tip; 50-μm tip diameter), placed at either side of the recording pipette, hereby stimulating nonoverlapping populations of the Schaffer collateral-associational commissural pathway. For baseline recordings, stimulation intensity was adjusted to values evoking a potential slope of approximately 25 to

30% of the maximum response. After at least 20 min of stable baseline recordings, long-term potentiation was induced by delivering a high-frequency stimulation train (100 pulses delivered at 100 Hz for 1 s) *via* one of the stimulating electrodes. The use of both stimulating electrodes allowed the measurement of an internal control in the same slice. After high-frequency stimulation was delivered from one of the electrodes in the absence of any substance, potentiation of the responses was monitored for at least 60 min after the tetanic stimulus, maintaining the same settings used for the baseline. Then, midazolam was applied in the bath solution for 60 min before long-term potentiation induction in the second input after high-frequency stimulation delivered *via* the second electrode. Inhibition of long-term potentiation was defined when the potential's slope after high-frequency stimulation was less than 120% of the prestimulation slope. Control experiments corroborate that the extent of long-term potentiation was independent during the time that slices were in the recording chamber, at least for the duration in the current studies (up to 5 h). The field potentials were amplified (BA-2S, npi electronic, Germany), filtered (3 kHz), and digitized (9 kHz) using a laboratory interface board (ITC-16, Instrutech Corp., USA) and the WinLTP program software,[24] available at http://www.winltp.com/Ltp24/indexLtp24.htm. Stimuli were administered in an alternating manner to each input every 15 s. Two signals from the respective input were averaged to one for analysis, representing one data point each minute. Offline reanalysis of the data was performed with the same WinLTP software. The field excitatory postsynaptic potential slope was measured between 20 and 80% of the peak amplitude and then normalized according to the 20-min baseline recording before tetanic stimulation. The slope is commonly measured (instead of the amplitude) because it is a less contaminated signal and therefore more reliable.

Patch Clamp Measurement of Synaptic Currents in Cultured Neocortical Tissue and Hippocampal Tissue Slices

The whole cell configuration of the patch clamp technique was used for analyzing midazolam's effects on $GABA_A$ receptor–mediated synaptic currents in cortical neurons. Cultured neocortical tissue slices showing signs of damage and degradation were excluded from electrophysiologic experiments. Intact neocortical slices were placed in a heated bath chamber (34°C) and continuously perfused with a modified artificial cerebrospinal fluid at a flow rate of 1 ml/min. The perfusion fluid consisted of 120 mM NaCl, 3.5 mM KCl, 1.1 mM NaH_2PO_4, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 1.2 mM $CaCl_2$, and 11 mM D-glucose and was bubbled with 95% O_2/5% CO_2 to adjust the pH to 7.4. Glass pipettes (2.5- to 4.5-MΩ tip resistance with recording solution) were pulled from borosilicate glass (World Precision Instruments, USA) with a puller (P-2000, Sutter Instrument Company, USA) and filled with high-chloride intracellular solution containing 121 mM CsCl, 24 mM CsOH, 1 mM $MgCl_2$, 5 mM EGTA, 10 mM HEPES, and 4 mM adenosine triphosphate. The recording electrode was positioned on a neuron with a micromanipulator, using infrared imaging. The cells were held at −70 mV in the whole cell voltage clamp configuration to record miniature synaptic events in the cultured neocortical slices. The *N*-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor antagonists D-2-amino-5-phosphonopentanoate (50 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM) or 2,3-dioxo-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (5 μM; all Sigma-Aldrich) were added to the extracellular bath solution to block glutamatergic currents.

Quantification of the kinetic properties of synaptic currents requires events that do not overlap with others. This kind of event was rare when monitoring action potential–dependent and –independent GABAergic inhibitory postsynaptic currents together in cultured neocortical slices. Therefore, the sodium-channel blocker tetrodotoxin was added for blocking action potential dependent events. Currents were amplified with a MultiClamp 700A amplifier; sampled and digitized at a frequency of 20 kHz with a Digidata 1440A digitizer and Clampex 10.4 software (Molecular Devices, USA); and stored on a computer. Control recordings (duration, 180 s) were started after a 6-min wash-in phase with the blockers. Then the drug (50 or 500 nM midazolam) was added to the bath solution, and the drug recording (180 s) was started after 15 min. Offline processing of digitized data was performed using the MATLAB R2018b (The MathWorks Inc., USA) program package. Digitized current recordings in abf format were imported, and synaptic events were detected by setting a threshold that was five times higher than the baseline noise (SD of the baseline current in the absence of events). Recordings with unstable baselines were excluded. Each experimental group contained recordings with cultures from at least two different preparations. For these experiments, the first n value represents the number of successful recordings, whereas the second one reports the number of animals.

In another set of experiments, we explored midazolam's effect on GABAergic synaptic currents in acutely isolated hippocampal slices derived from wild-type animals. Because the frequency of spontaneously occurring synaptic currents was considerably smaller in this preparation than in cultured neocortical slices, action potential–dependent and –independent events were monitored together for evaluating midazolam's effect on this type of neurons. Signals were amplified with the npi SEC-10L amplifier and digitized with HEKA LIH8+8 acquisition system and the PatchMaster v2x90.3 (HEKA Elektronik GmbH, Germany) software. For monitoring spontaneous synaptic events, control recordings were performed after assuring the health of the cell and then continued after 35 min of washing with midazolam 100 nM.

We opted for a longer drug exposure time in acutely isolated hippocampal slices than in cultured neocortical slices because the former were much thicker (acutely isolated hippocampal slices, 350 μm; in cultured neocortical slices, the initial thickness of about 300 μm is reduced to less than 30 μm after 2 weeks in culture), and the diffusion of drugs into the tissue is slower.[25] In all recording sessions, only a single concentration measurement was performed. After exposure to midazolam, the tissue slices were excluded from further experiments.

Statistical Analysis of Long-term Potentiation Experiments

No explicit randomization or blinding methods were used for the assignment of individual animals to experimental conditions. All data values were normally distributed and are presented as the mean ± SD. For extracellular recordings, the n value is shown as *x* slices from *y* animals as, for example, n = 12/8, with the first number the number of slices and the second the total number of animals used in those conditions. The sample size was determined based on previous experience, and a maximum of two slices per animal were used, assuming that these slices are independent within animals. No statistical difference was found between male and female mice within genotype (Supplemental Digital Content 1, table S2, http://links.lww.com/ALN/C845). For all long-term potentiation experiments, comparisons of outcomes from three or more groups were performed using one-way repeated-measures ANOVA analysis with a 95% CI followed by Dunnett's *post hoc* test to correct for multiple comparisons. We analyzed the long-term potentiation experiments containing two groups with the paired Student's *t* test.

Statistical Analysis of Voltage Clamp Recordings of Synaptic Currents

A standard recording interval of 3 min delivered several hundred miniature spontaneous events in cultured neocortical slices. These events were individually inspected and eliminated from the analysis if they overlapped with others. Events sampled in the course of a single recording that fulfilled the latter condition were fitted with a biexponential equation in the form $I(t) = A_f \exp(-t/\tau_{fast}) + A_s \exp(-t/\tau_{slow}) + c$, where I(t) is the current amplitude at any given time t, c is the baseline current, τ_{fast} and τ_{slow} are the fast and slow time constants of current decay, and A_f and A_s are the estimated fast and slow intercepts of the components at time zero,[26–28] respectively. Weighted time constants were calculated using the following equation: $\tau_w = A_f/(A_f + A_s) \star \tau_{fast} + A_s/(A_f + A_s) \star \tau_{slow}$. Midazolam's effects on this parameter were visualized by plotting histograms, which were well fitted with a double Gaussian function (fig. 5B; Supplemental Digital Content 1, fig. 1B, http://links.lww.com/ALN/C845): $val(x)=a1 \star e^{-\frac{(x-b1)}{c1^2}} + a2 \star e^{-\frac{(x-b2)}{c2^2}}$ with a1, a2 = max1, max2; b1, b2 = mean1, mean2; and $c1 \cdot \sqrt{2}$, $c2 \cdot \sqrt{2}$ = SD.

For further statistical analysis of the kinetic properties of synaptic events, we used the weighted decay time, the amplitude ($A_f + A_s$), and the charge transferred per miniature event. The Lilliefors test indicated that these parameters were normally distributed. For curve fitting and further statistical analysis, the program package MATLAB R218b was used. From the fits of individual synaptic currents captured in the same recording, the mean amplitude, the mean of the weighted decay time, and the mean transferred charge per event were calculated. These means provided an estimation of the kinetic properties of synaptic currents obtained from one individual experiment. Results from different experiments were used for comparing different genotypes and different midazolam concentrations in a final step (fig. 6).

In a subset of experiments, the kinetic properties of synaptic currents were estimated by using a different analytical approach in addition to the method described above, which served as an internal control. In this case, synaptic events that were nonoverlapping and detected in the course of a single recording were aligned by the point in time the current crossed the detection threshold for the first time (fig. 4). Aligned synaptic currents were stored in a two-dimensional matrix with the dimension time on the *x*-axis and the running number of detected events on the *y*-axis. Next, the median current was derived from various events and was determined for all points in time, providing an estimation of the time course of the median synaptic event. We calculated medians instead of means because statistical distributions were *a priori* unknown. In a final step, the time course of this median synaptic event was fitted with a double-exponential function as detailed above (fig. 5A). Results obtained with this alternative way of quantifying kinetic properties of synaptic events confirmed the results provided by our standard method.

Lilliefors tests indicated a normal distribution of kinetic parameters that were estimated from various experiments. For the group comparison of the genotypes (wild-type *vs.* $\alpha_{2/3/5}$–knock-in) at 50 or 500 nM midazolam, as well as the comparison of the concentrations (50 *vs.* 500 nM midazolam), we performed independent sample *t* tests, whereas for the comparison control *vs.* drug, a paired samples *t* test (due to the paired design of the experiments) was sufficient.

Individual spontaneous inhibitory postsynaptic currents in acutely isolated hippocampal slices were analyzed with mini analysis software (Synaptosoft, USA). The detection threshold was set five times higher than the SD of the baseline current in the absence of events, and data on decay time and amplitude were collected. Curve fitting and further statistical analysis were conducted with GraphPad Prism 6.01 (GraphPad Software, USA) applying equations and statistical tests as described above. Differences were considered significant when the two-tailed $P < 0.05$ and are indicated by asterisks.

Results

Midazolam Depresses Hippocampal Long-term Potentiation at Low Nanomolar Concentrations (Greater than 3 nM)

First, we determined the effect of several concentrations of midazolam on the induction of long-term potentiation in the CA1 hippocampal region (fig. 1). In the control group, field excitatory postsynaptic potentials were potentiated to 156 ± 19% (n = 20/12, 8 male and 4 female mice). After 60 min of midazolam (3 nM) exposure, long-term potentiation was induced by administering a tetanic stimulation resulting in a potentiation of 150 ± 25% (n = 6/3, all males) that was not significantly depressed compared to that of the control group. In contrast, midazolam applied at 10 nM (fig. 1B) and 1 μM, but not at 3 nM, resulted in a significant blockage of long-term potentiation for 10 nM at 98 ± 11% (n = 14/8, 4 male and 4 female mice) and for 1 μM at 94 ± 17% (n = 6/5, all males), indicating a dose-dependent effect (fig. 1C; Supplemental Digital Content 1, table S3A, http://links.lww.com/ALN/C845).

Flumazenil Can Prevent the Action of Midazolam

After demonstrating that midazolam at 10 nM was blocking long-term potentiation, we were interested to know whether this effect is mediated *via* the classical benzodiazepine site. Accordingly, we applied the specific benzodiazepine-site antagonist flumazenil[28] in the presence of midazolam to prevent this action. After the previous application of midazolam, flumazenil was able to significantly block inhibition of long-term potentiation by midazolam (midazolam, 113 ± 8%, n = 10/5, all males *vs.* midazolam + flumazenil, 145 ± 17%, n = 10/5, all males; fig. 2; Supplemental Digital Content 1, table S3B, http://links.lww.com/ALN/C845) and did not display any intrinsic effect when applied alone at 30 nM (data not shown).

α_1-GABA$_A$ Receptor Subunit Plays a Major Role in Midazolam's Effect

The next set of experiments was designed to explore the role of the different GABA$_A$ receptor subunits in the effect of midazolam by using several transgenic mouse models, where one or three α-subunits were carrying an H-to-R

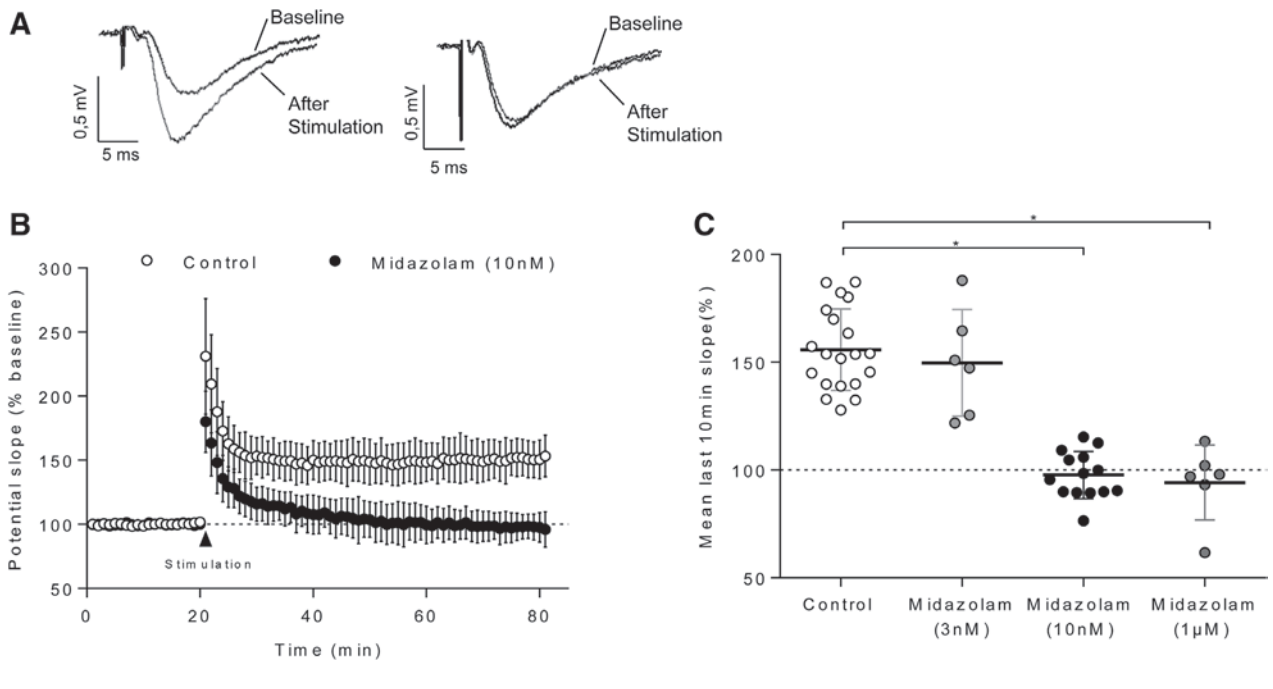

Fig. 1. Effects of midazolam after long-term potentiation induction in the hippocampal CA1 region. (*A*) Raw traces of field excitatory postsynaptic potential recorded under control conditions (*left*) and in 10 nM midazolam (*right*). (*B*) Time course of the induction/maintenance of long-term potentiation after the tetanic stimulation in a control experiment (n = 20/12 [n = slices from animals], 8 male and 4 female mice) and after the perfusion of 10 nM midazolam (n = 14/8, 4 male and 4 female mice). Field excitatory postsynaptic potential slopes (mean ± SD) and all responses were normalized to the baseline, which was stable for 20 min before the delivery of the high-frequency stimulation (*arrow* in the graph). (*C*) Scatter plot summarizing the last 10 min after high-frequency stimulation showing that 3 nM midazolam did not alter long-term potentiation (150 ± 25%, n = 6/3, all males *vs.* control 156 ± 19%, n = 20/12), but 10 nM (98 ± 11%, n = 14/8) and 1 μM (94 ± 17%, n = 6/5, all males) significantly blocked long-term potentiation in a dose-dependent manner. One-way repeated-measures ANOVA followed by Dunnett's *post hoc* test showed significant differences between control and midazolam concentrations of 10 nM and 1 μM but not for the 3 nM concentration (Supplemental Digital Content 1, table S3A, http://links.lww.com/ALN/C845).

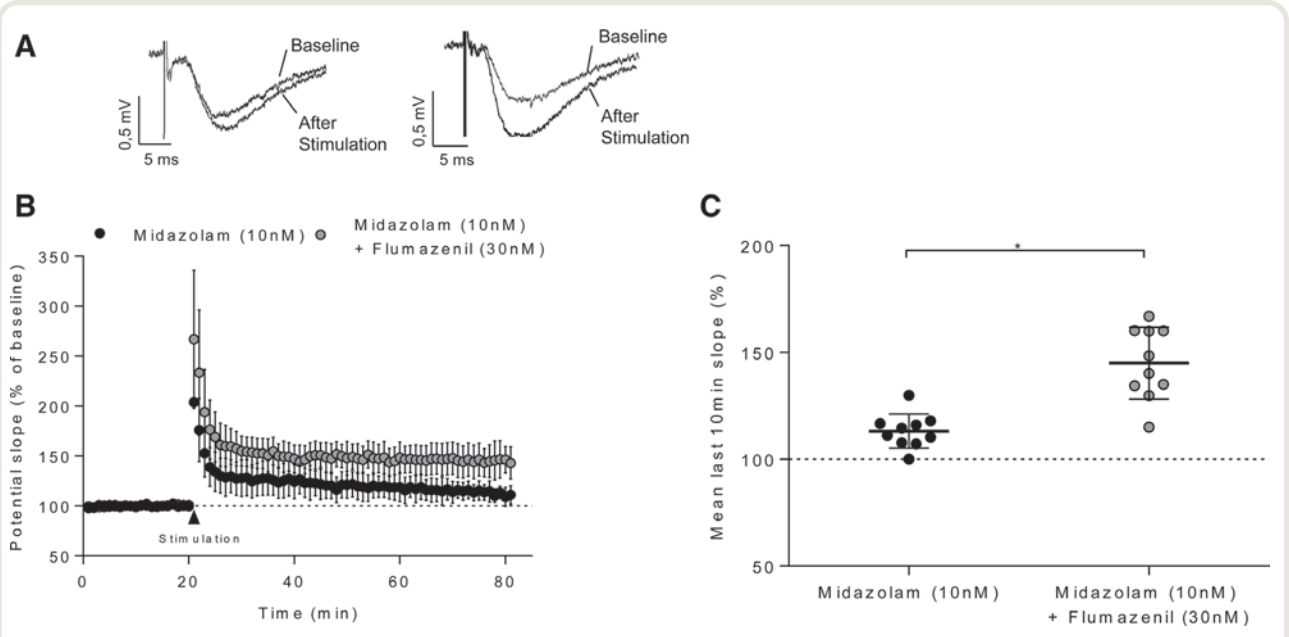

Fig. 2. Effects of midazolam and the antagonist flumazenil after long-term potentiation induction in the hippocampal CA1 area. (*A*) Raw traces of field excitatory postsynaptic potentials in the presence of 10 nM midazolam (*left*) and during coapplication of midazolam and flumazenil (*right*). (*B*) Time course of the induction/maintenance of long-term potentiation after tetanic stimulation when the slices were pretreated with midazolam 10 nM and after the addition of flumazenil 30 nM. Field excitatory postsynaptic potential slopes (mean ± SD) and all responses were normalized to the baseline, which were stable 20 min before the delivery of the high-frequency stimulation (*arrow* in the graph). (C) The scatter plot of averaged values of the last 10 min after high-frequency stimulation shows that preadministration of 10 nM midazolam blocked long-term potentiation (113 ± 8%, n = 10/5 [n = slices from animals], all males), but when its antagonist flumazenil is perfused at a concentration three times higher, this was able to prevent the long-term potentiation blocking effect that was caused by the previous midazolam (145 ± 17%, n = 10/5, all males). A paired *t* test showed significant differences between the first application of 10 nM midazolam and the additional perfusion of flumazenil (Supplemental Digital Content 1, table S3B, http://links.lww.com/ALN/C845).

knock-in mutation. We investigated midazolam's actions on long-term potentiation evoked in hippocampal brain slices in which α_1-, α_2-, α_5-, $\alpha_{1/2/3}$-, $\alpha_{1/3/5}$-, and $\alpha_{2/3/5}$-subunit–containing receptors are insensitive to midazolam. Because of its low expression in the hippocampus,[29] we did not focus on α_3.

When high-frequency stimulation was administered in α_1–knock-in mice in the presence of 10 nM midazolam (fig. 3A; Supplemental Digital Content 1, table S3C, http://links.lww.com/ALN/C845), long-term potentiation was not significantly altered (136 ± 9% *vs.* control 141 ± 9%, n = 18/13, 8 male and 5 female mice), while a significant long-term potentiation blockage was observed for the $\alpha_{2/3/5}$–knock-in (fig. 3B; Supplemental Digital Content 1, table S3D, http://links.lww.com/ALN/C845; 106 ± 12% *vs.* control 167 ± 27%, n = 12/6, 3 male and 3 female mice), α_5–knock-in (fig. 3C; Supplemental Digital Content 1, table S3E, http://links.lww.com/ALN/C845; 106 ± 17% *vs.* control 143 ± 12%, n = 20/15, 10 male and 5 female mice), and $\alpha_{1/2/3}$–knock-in lines (fig. 3D; Supplemental Digital Content 1, table S3F, http://links.lww.com/ALN/C845; 101 ± 21% *vs.* control 148 ± 10%, n = 13/11, 9 male and 2 female mice). In contrast, long-term potentiation in slices of $\alpha_{1/3/5}$–knock-in mice (fig. 3E; Supplemental Digital Content 1, table S3G, http://links.lww.com/ALN/C845; 141 ± 10% *vs.* control 139 ± 12%, n = 12/6, 6 male and 6 female mice) was not significantly altered in the presence of 10 nM midazolam. These results show that the effect of midazolam at small concentrations in wild-type mice is mainly mediated by the α_1-subunit (fig. 3, A and B). However, when this subunit is rendered insensitive to benzodiazepines by a knock-in point mutation, it is obvious that the combined action of midazolam on α_2- or α_5-subunits is unable to block long-term potentiation. Interestingly, long-term potentiation in the $\alpha_{1/2/3}$–knock-in genotype (with an intact α_5) is midazolam-sensitive (fig. 3D).

Midazolam Modulates α_1-Receptors in Neocortical Slice Cultures

In a second series of experiments, we investigated the effects of midazolam on GABAergic synaptic transmission, selectively mediated *via* α_1-$GABA_A$ receptors. To this end, we recorded miniature synaptic currents in organotypic slice cultures prepared from the neocortex of wild-type and $\alpha_{2/3/5}$–knock-in mice under drug-free conditions and

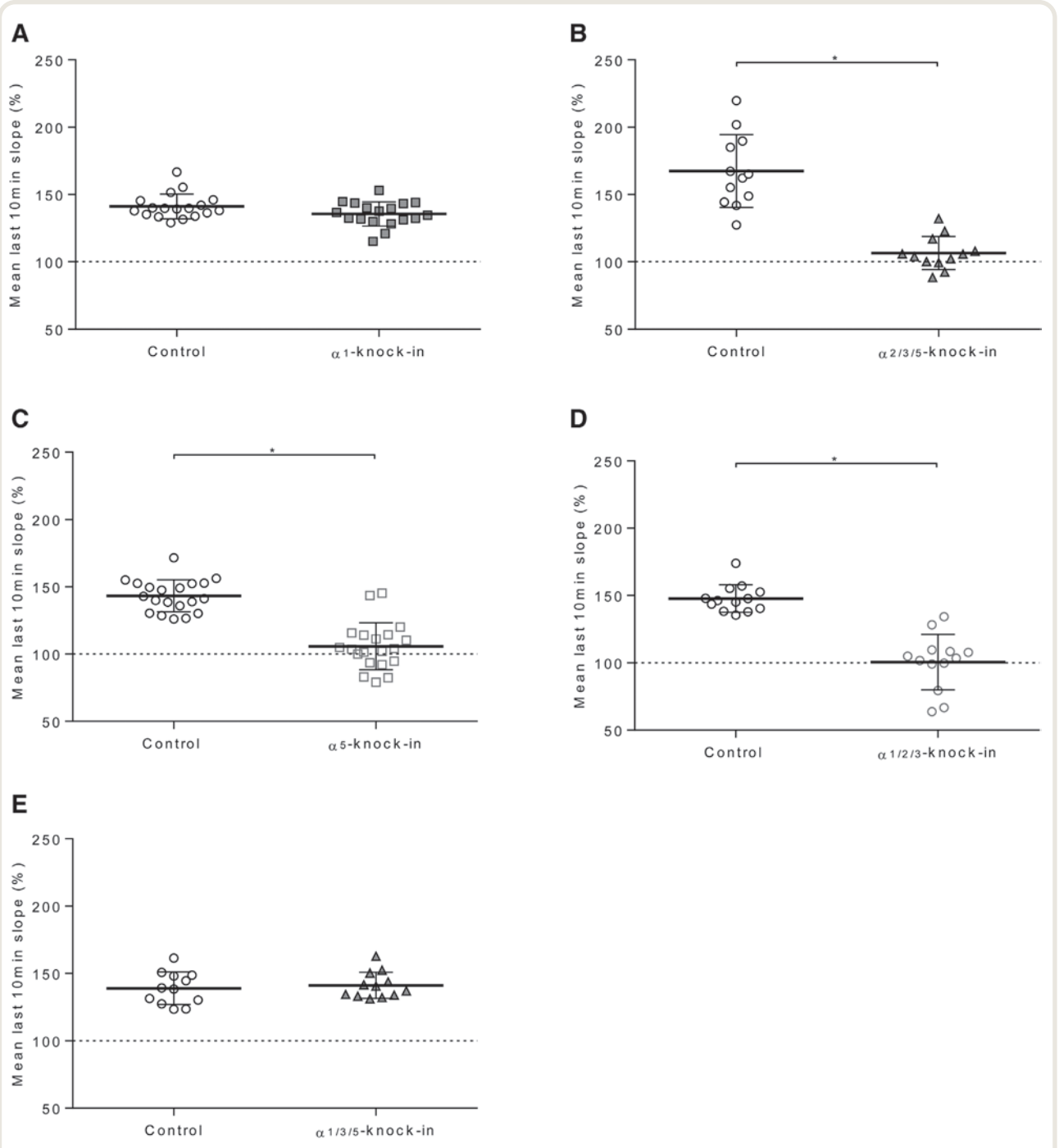

Fig. 3. Effect of 10 nM midazolam after long-term potentiation in α_1–knock-in, $\alpha_{2/3/5}$–knock-in, α_5–knock-in, $\alpha_{1/2/3}$–knock-in, and $\alpha_{1/3/5}$–knock-in mice. Field excitatory postsynaptic potential slopes of the last 10 min of the high-frequency stimulation show that in α_1–knock-in genotype (*A*), long-term potentiation is not altered (midazolam 136 ± 9% *vs.* control 141 ± 9%, n = 18/13 [n = slices from animals], 8 male and 5 female mice). In $\alpha_{2/3/5}$–knock-in (*B*; midazolam 106 ± 12% *vs.* control 167 ± 27%, n = 12/6, 3 male and 3 female mice), α_5–knock-in (*C*; midazolam 106 ± 17% *vs.* control 143 ± 12%, n = 20/15, 10 male and 5 female mice), and $\alpha_{1/2/3}$–knock-in (*D*) genotypes (midazolam 101 ± 21% *vs.* control 148 ± 10%, n = 13/11, 9 male and 2 female mice), a blockage of long-term potentiation was seen after the perfusion of 10 nM midazolam. Long-term potentiation on $\alpha_{1/3/5}$–knock-in mice (*E*; midazolam 141 ± 10% *vs.* control 139 ± 12%, n = 12/6, 6 male and 6 female mice) was not significantly altered (Supplemental Digital Content 1, table S3, C to G, http://links.lww.com/ALN/C845).

in the presence of 50 or 500 nM midazolam. Spontaneous events recorded under drug-free conditions served as the control. Then midazolam (50 nM or 500 nM) was added, and spontaneous events were recorded under drug conditions. A representative experiment is displayed in figure 4, showing exemplary current traces (left) and the overlaid miniature synaptic events sampled from the same recording (right) under control conditions (fig. 4A) and after application of midazolam (fig. 4B) in slices of wild-type mice. In Supplemental Digital Content 1, figure S2 (http://links.lww.com/ALN/C845), the same kind of experiment using slices of $\alpha_{2/3/5}$–knock-in mice is indicated. Figure 5 and Supplemental Digital Content 1, figure S1 (http://links.lww.com/ALN/C845) demonstrate the impact of midazolam on the kinetic properties of miniature synaptic events. Individual synaptic events monitored in the absence and presence of midazolam were fitted with biexponential functions. The median synaptic event obtained from synaptic events monitored in the presence of midazolam was normalized by the maximum current of the median synaptic event monitored under drug-free conditions. Midazolam clearly increased decay time in neocortical neurons of wild-type mice (fig. 5A) and $\alpha_{2/3/5}$–knock-in mice (Supplemental Digital Content 1, fig. S1A [http://links.lww.com/ALN/C845]). Histograms in figure 5B and Supplemental Digital Content 1, figure S1B (http://links.lww.com/ALN/C845) display the weighted decay times of individual miniature events, sampled during the same

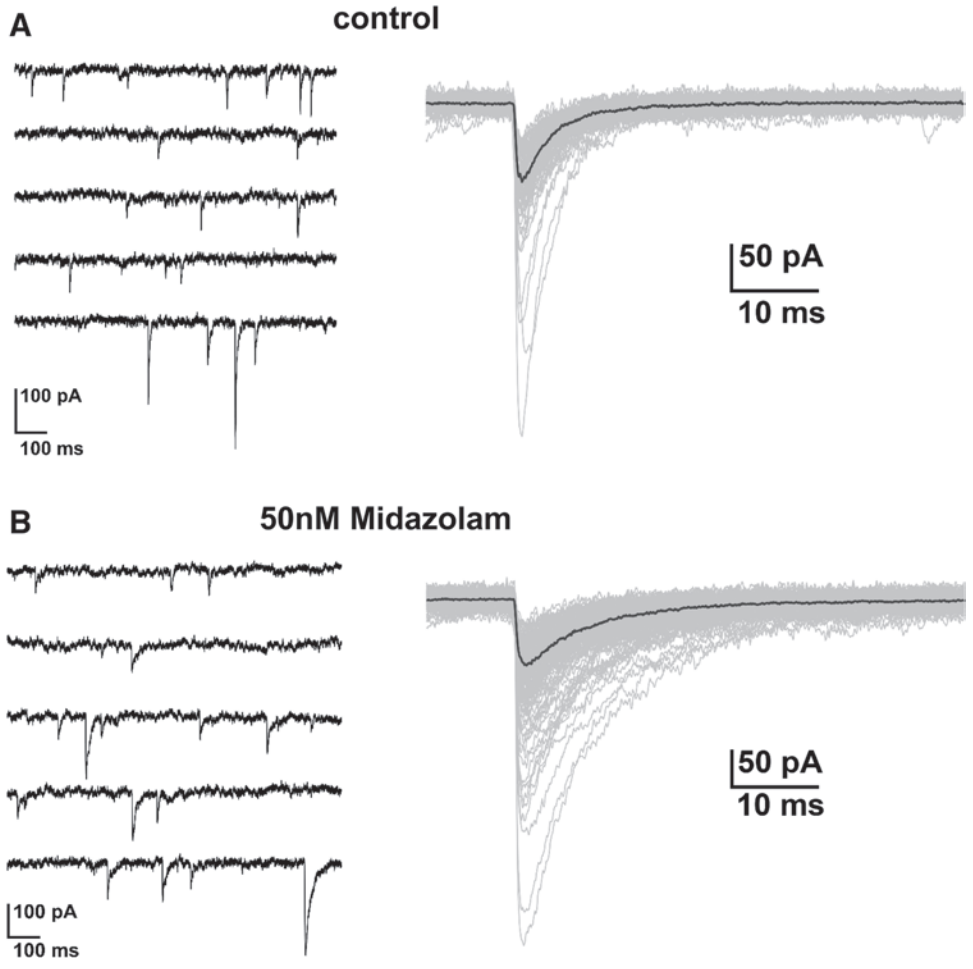

Fig. 4. Representative experiment: spontaneous γ-aminobutyric acid type A ($GABA_A$) receptor-mediated miniature synaptic events in voltage clamped neocortical neurons from wild-type mice held at a membrane potential of –70 mV. The recordings were carried out on the same neuron in the presence of tetrodotoxin and high internal chloride concentration, before midazolam application (*A*) and in the presence of 50 nM midazolam (*B*). (*Left*) Representative current traces are shown. (*Right*) A total of 80 consecutive synaptic events were extracted from these recordings and are displayed overlayed. The *gray traces* represent the single synaptic events, whereas the *black traces* represent the median miniature synaptic event (median of these 80 consecutive synaptic events). Synaptic events were fitted with a biexponential function (see "Materials and Methods"). For fit parameters, see Supplemental Digital Content 1, table S4 (http://links.lww.com/ALN/C845). The midazolam treatment slowed the decay of γ-aminobutyric acid–mediated synaptic events but had little effect on their amplitude and frequency.

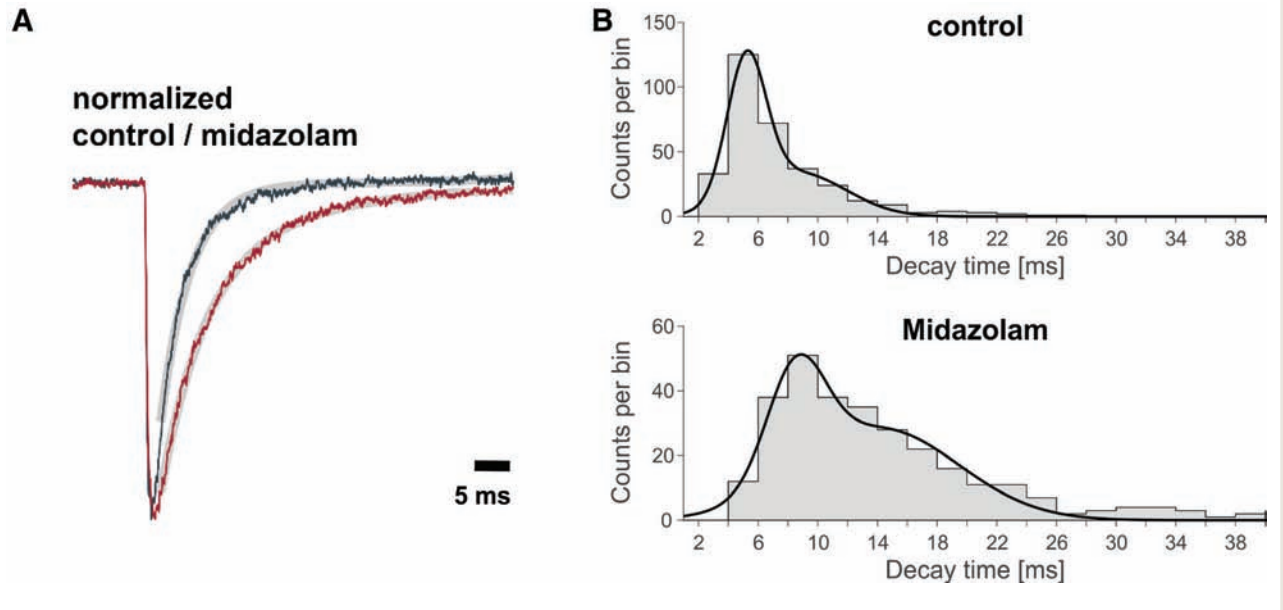

Fig. 5. Kinetic properties of miniature synaptic events in wild-type neocortical neurons in the absence and presence of midazolam. Individual synaptic events, monitored in the absence (control: n = 326) and presence of midazolam (n = 291) were fitted with biexponential functions. (*A*) Median synaptic event of control recording (*black trace*) and after treatment with midazolam (*red trace*). The drug recording was normalized by the maximum current of the control recording. Weighted decay times were as follows: $\tau_{weighted/control}$ of 3.91 ms and $\tau_{weighted/drug}$ of 8.89 ms. For detailed fit parameters, see Supplemental Digital Content 1, table S5A (http://links.lww.com/ALN/C845). (*B*) The weighted decay time constants of miniature postsynaptic currents, derived from biexponentials fits of individual synaptic events (control: n = 326, midazolam: n = 291), were binned into histograms, using a bin width of 2 ms (*gray area*). Distributions of weighted decay times were fitted by the sum of two Gaussian functions (see "Materials and Methods"). These two distributions do not correlate with the fast and slow time constants of biexponential fits but indicate the existence of distinct populations of miniature synaptic events, featuring different kinetic properties. Midazolam almost doubled the means of Gaussian functions. For estimated fit parameters, see Supplemental Digital Content 1, table S5B (http://links.lww.com/ALN/C845).

recording interval. Distributions were best fitted by the sum of two Gaussian functions, suggesting the presence of at least two distinct populations of synaptic events before and during midazolam treatment.

Next, we analyzed midazolam effects on frequency, charge transfer, amplitude, and decay time of synaptic events and compared the effects of two different concentrations (50 and 500 nM) and genotypes (wild-type and $\alpha_{2/3/5}$–knock-in mice; fig. 6; Supplemental Digital Content 1, fig. S3, http://links.lww.com/ALN/C845). We did not observe significantly differing effects on the frequency between genotypes and drug concentrations (data not shown). Midazolam clearly increased the charge transferred per synaptic event (fig. 6A; Supplemental Digital Content 1, table S6A1, http://links.lww.com/ALN/C845), and the effect was significant for both genotypes and drug concentrations. Midazolam (50 nM) increased charge transfer equally in wild-type and $\alpha_{2/3/5}$–knock-in mice (fig. 6, A1 and A2): 50 nM *versus* control in wild-type: 172 ± 71% of control; 50 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: 171 ± 62% of control (Supplemental Digital Content 1, table S6, A2.1 and A2.2, http://links.lww.com/ALN/C845). Interestingly, increasing the concentration of midazolam further increased charge transfer in the wild-type but not in the $\alpha_{2/3/5}$–knock-in cultures (fig. 6, A1 and A2; Supplemental Digital Content 1, table S6, A2.1 and A2.2, http://links.lww.com/ALN/C845): 500 nM midazolam in wild-type: 236 ± 54% of control, n = 6/6; 500 nM midazolam in $\alpha_{2/3/5}$–knock-in: 175 ± 62% of control, n = 7/6. Since there is no difference in the effect size between wild-type and $\alpha_{2/3/5}$–knock-in mice at 50 nM midazolam, the effect on charge transfer at this concentration seems to be largely mediated by α_1-$GABA_A$ receptors. Additional experiments were added in response to peer review in which midazolam's action on GABAergic currents was determined in the presence of the benzodiazepine site antagonist flumazenil. With flumazenil present, 500 nM midazolam did not cause a change in charge transfer that was statistically significant (107 ± 13% of control; n = 5/3; all wild-type).

The charge transfer per synaptic event is primarily determined by two components: amplitude and decay time of synaptic events. By analyzing the effect on these parameters, we can infer their relative contribution to the concentration-dependent effect on charge transfer in wild-type cultures.

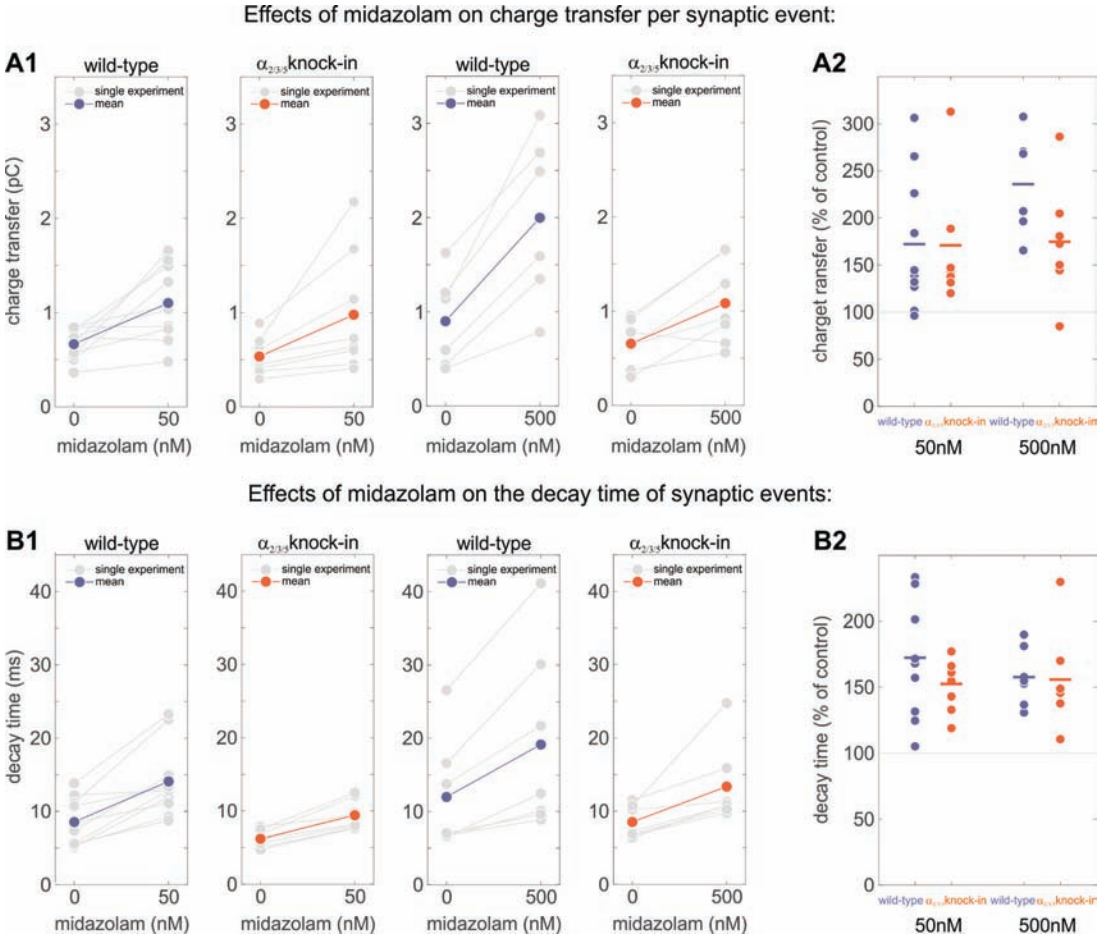

Fig. 6. Effect of 50 and 500 nM midazolam on charge transfer (*A1* and *A2*) and weighted decay time (*B1* and *B2*) of miniature synaptic events in wild-type (*blue*) and $\alpha_{2/3/5}$–knock-in mice (*orange*). (*A1* and *B1*) Individual experiments (paired design: control and drug recording) in wild-type and $\alpha_{2/3/5}$–knock-in. The plots display the absolute values of charge transfer (pC) and decay time (ms) of miniature synaptic events before (0 nM = control) and after application of 50 or 500 nM midazolam. The connected dots in *gray* show single experiments, whereas the *colored traces* represent the mean values. *A1* and *B1* are composed of four subplots that display the effects (from *left* to *right*) of 50 nM midazolam in wild-type, 50 nM in $\alpha_{2/3/5}$–knock-in, 500 nM in wild-type, and 500 nM in $\alpha_{2/3/5}$–knock-in on the respective parameters. (*A1*) Midazolam 50 and 500 nM significantly increased charge transfer per synaptic event in wild-type and $\alpha_{2/3/5}$–knock-in (50 nM *vs.* control in wild-type: mean difference = +0.44 ± 0.40 [pC], n = 10/6; 50 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: mean difference = +0.44 ± 0.48 [pC], n = 8/6; 500 nM *vs.* control in wild-type: mean difference = +1.01 ± 0.51 [pC], n = 6/6; 500 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: mean difference = +0.43 ± 0.32 [pC], n = 7/6; Supplemental Digital Content 1, table S6A1, http://links.lww.com/ALN/C845). (*B1*) Midazolam 50 and 500 nM significantly increased the decay time of synaptic events in wild-type and $\alpha_{2/3/5}$–knock-in (50 nM *vs.* control in wild-type: mean difference = +5.54 ± 3.44 ms, n = 10/6; 50 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: mean difference = +3.21 ± 1.36 ms, n = 8/6; 500 nM *vs.* control in wild-type: mean difference = +7.15 ± 5.12 ms, n = 7/6; 500 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: mean difference = +4.82 ± 4.19 ms, n = 7/6; Supplemental Digital Content 1, table S6B1, http://links.lww.com/ALN/C845). (*A2* and *B2*) Comparison of the relative drug effect (normalized to the respective control; the *line* at 100% indicates the effect size of the control recordings) of 50 nM (*left*) and 500 nM (*right*) midazolam in wild-type (*blue*) and $\alpha_{2/3/5}$–knock-in (*orange*) on charge transfer (*A2*) and decay time (*B2*) of synaptic events. (*A2*) Midazolam increased the charge transferred per synaptic event. The effect was significant for both genotypes and drug concentrations (Supplemental Digital Content 1, table S6A2.1, http://links.lww.com/ALN/C845). Midazolam (50 nM) increased charge transfer almost equally in wild-type and $\alpha_{2/3/5}$–knock-in (50 nM *vs.* control in wild-type: 172 ± 71% of control; 50 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: 171 ± 62% of control [Supplemental Digital Content 1, table S6A2.2, http://links.lww.com/ALN/C845]; wild-type *versus* $\alpha_{2/3/5}$–knock-in at 50 nM: not significant [Supplemental Digital Content 1, table S6A2.1, http://links.lww.com/ALN/C845]). Increasing the concentration of midazolam further increased the charge transfer in the wild type, but not in the $\alpha_{2/3/5}$–knock-in (500 nM midazolam in wild-type: 236 ± 54% of control; 500 nM midazolam in $\alpha_{2/3/5}$–knock-in: 175 ± 62% of control; Supplemental Digital Content 1, table S6, A2.1 and A2.2, http://links.lww.com/ALN/C845). (*B2*) Midazolam 50 and 500 nM significantly prolonged the decay of synaptic events in wild-type and $\alpha_{2/3/5}$–knock-in mice (50 nM *vs.* control in wild-type: 172 ± 71% of control, n = 10/6; 500 nM *vs.* control in wild-type: 158 ± 21% of control, n = 7/6; 50 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: 153 ± 19% of control, n = 10/6; 500 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: 156 ± 37%, n = 7/6). We did not find significant differences between the genotypes or a concentration-dependent effect on the decay time. For statistical comparisons, see Supplemental Digital Content 1, table S6B2.1 (http://links.lww.com/ALN/C845); for relative decay times, see Supplemental Digital Content 1, table S6B2.2 (http://links.lww.com/ALN/C845).

Midazolam at concentrations of 50 and 500 nM significantly prolonged the decay of miniature synaptic events (fig. 6, B1 and B2) in wild-type and $\alpha_{2/3/5}$–knock-in slice cultures (50 nM *vs.* control in wild-type: 172 ± 44% of control, n = 10/6; 500 nM *vs.* control in wild-type: 158 ± 21% of control, n = 7/6; 50 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: 153 ± 19% of control, n = 10/6; 500 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: 156 ± 37%, n = 7/6; Supplemental Digital Content 1, table S6, B1, B2.1, and B2.2, http://links.lww.com/ALN/C845). However, we did not find significant differences between the genotypes or a concentration dependency on the decay time of miniature synaptic events (fig. 6B2; Supplemental Digital Content 1, table S6B2.1, http://links.lww.com/ALN/C845).

The amplitude of synaptic events (Supplemental Digital Content 1, fig. S3A, http://links.lww.com/ALN/C845) was not significantly altered by 50 nM midazolam in wild-type and $\alpha_{2/3/5}$–knock-in mice (Supplemental Digital Content 1, fig. S3, A and B, http://links.lww.com/ALN/C845; 50 nM *vs.* control in wild-type: 104 ± 27%, n = 10/6; 50 nM *vs.* control in $\alpha_{2/3/5}$–knock-in: 120 ± 30%, n = 8/6). Midazolam (500 nM) did not change the amplitude of synaptic events in the $\alpha_{2/3/5}$–knock-ins (123 ± 35%, n = 7/6; Supplemental Digital Content 1, fig. S3, A and B, http://links.lww.com/ALN/C845) but significantly increased the amplitude of synaptic events in the wild type (137 ± 36%, n = 7/6; Supplemental Digital Content 1, fig. S3, A and B, http://links.lww.com/ALN/C845). We did not observe a significant difference in the amplitude of synaptic events between the genotypes (Supplemental Digital Content 1, fig. 3B, http://links.lww.com/ALN/C845). Analogous to the effect on charge transfer, there was, however, a significant concentration dependency in the wild type (Supplemental Digital Content 1, fig. S3B, http://links.lww.com/ALN/C845). Therefore, the concentration-dependent increase of charge transferred per synaptic event observed in the wild type most likely results from an increase in the amplitude of synaptic events only occurring at high concentrations of midazolam. Since the effects of midazolam at low concentrations seem to be almost exclusively mediated *via* α_1-$GABA_A$ receptors but did not further increase in the $\alpha_{2/3/5}$–knock-ins at a higher concentration of midazolam, the additional effects of midazolam at higher concentrations observed in the wild type are possibly attained by modulating the amplitude of synaptic events *via* $GABA_A$ receptor subpopulations without an α_1-subunit.

Synaptic Currents in Acute Brain Hippocampal Slices

In CA1 hippocampal neurons from wild-type mice, spontaneous synaptic events followed the same pattern as in neocortical slices from wild-type animals. A significant increase in the decay time was seen after application of 100 nM midazolam (35 ± 1% *vs.* control 31 ± 3%, n = 10/6, all males; Supplemental Digital Content 1, fig. S4A, http://links.lww.com/ALN/C845). Similarly, the amplitude of the spontaneous synaptic events was significantly increased after application of 100 nM midazolam (Supplemental Digital Content 1, fig. S4B, http://links.lww.com/ALN/C845; 46 ± 15% *vs.* control 31 ± 12%, n = 10/6, all males).

Discussion

Our results suggest that at amnestic concentrations (approximately 10 nM), midazolam blocks hippocampal long-term potentiation predominantly by potentiating α_1-$GABA_A$ receptors. We hypothesize that midazolam targeting α_2-$GABA_A$ receptors did not modulate long-term potentiation but dampened the activity of α_5-$GABA_A$ receptors. At hypnotic concentrations (50 to 500 nM), midazolam's enhancing effect on $GABA_A$ receptor–mediated synaptic transmission was largely mediated by α_1-$GABA_A$ receptors.

To elucidate midazolam's actions on hippocampal neurons, we utilized mouse lines carrying knock-in mutations in the α-subunit of the $GABA_A$ receptor, which cause a dramatic decrease in benzodiazepine binding.[9,18] The results summarized in figure 3 suggest that midazolam, which at low nanomolar concentrations causes moderate sedation and amnesia in humans, blocks hippocampal long-term potentiation predominantly *via* α_1-$GABA_A$ receptors. This conclusion is based on two complementary findings. First, in slices derived from $\alpha_{2/3/5}$–triple–knock-in mice, the drug was effective in blocking long-term potentiation. Second, midazolam failed to block long-term potentiation in slices derived from α_1–single–knock-in mice. Thus, modulation of α_1-receptors by midazolam seems to be sufficient to depress long-term potentiation. At first glance, these observations are surprising since previous studies showed that hippocampal-dependent long-term potentiation and learning are tightly controlled by α_5-receptors.[30,31] These receptors are densely expressed extrasynaptically on the dendrites of pyramidal cells, and their kinetic properties match functional properties of *N*-methyl-D-aspartate receptors.[32] In the current study, we also observed that in $\alpha_{1/2/3}$–triple–knock-in mice, midazolam was effective in depressing long-term potentiation, and MRK-016 (Supplemental Digital Content 1, fig. S5, http://links.lww.com/ALN/C845), a selective, negative modulator of α_5-receptors, enhanced long-term potentiation-induction in wild-type mice. Thus, selective modulation of α_5-receptors by midazolam and MRK-016 is effective in altering long-term potentiation. However, why does midazolam fail to depress long-term potentiation if the drug is a positive allosteric modulator of α_5- and α_2-receptors at the same time? This condition applies to slices derived from α_1–single–knock-in mice, since α_1-receptors are resistant to the drug in this genotype.[29] We hypothesize that the pool of α_2-receptors exerts inhibitory control over α_5-receptors. A possible scenario is that GABAergic interneurons, innervating the dendrites of hippocampal pyramidal cells, express α_2-receptors. As a consequence, concomitant modulation of α_5- and α_2-receptors reduces α_5-receptor function by

ultimately attenuating total GABA release onto pyramidal neurons (see fig. 7 for explanation). This hypothesis arises from comparing figure 3A (α_1–knock-in renders long-term potentiation midazolam-insensitive with α_2, α_3, and α_5 intact) with figure 3D ($\alpha_{1/2/3}$–knock-in renders long-term potentiation midazolam-sensitive with only α_5 intact). It is important to note that hypothesizing that the activity of α_5-receptors is dampened by α_2-receptors is compatible with midazolam's qualitative actions on all six genotypes investigated in the current study (fig. 7). Even though Crestani *et al.*[2] reported reduced α_5-receptor expression in the hippocampus of α_5–knock-in mice, they clearly showed that baseline functions (*e.g.*, long-term potentiation in α_5–knock-in) are unaffected by the knock-in mutation; this lets us conclude that these changes in α_5 receptor expression in knock-in mice may not interfere with our hypothesis. Of course, the idea of α_2-dependent α_5-receptor activity needs further corroboration, *e.g.*, monitoring neuronal activity within hippocampal inhibitory microcircuits of $\alpha_{1/3/5}$– and α_1–knock-in mice. In addition, the importance of α_1- and α_5-receptors involved in learning has been found also in rhesus monkeys.[33] Consistent with our hypothesis, in this study, α_1-receptors appear to be sufficient to induce learning impairments and thus to be dominant over α_5, as shown with the specific α_1 enhancers zolpidem and zaleplon.

Benzodiazepines induce multiple behavioral endpoints such as amnesia, anxiolysis, sedation, and hypnosis by acting on different sets of $GABA_A$ receptor subtypes that display diverging expression patterns in the central nervous system.[34] The drug's effective concentration is one crucial factor that defines these behavioral endpoints. Fortunately, in the case of midazolam, active metabolites are of minor importance,[22,35,36] and according to the calculation provided in the first supplementary table (Supplemental Digital

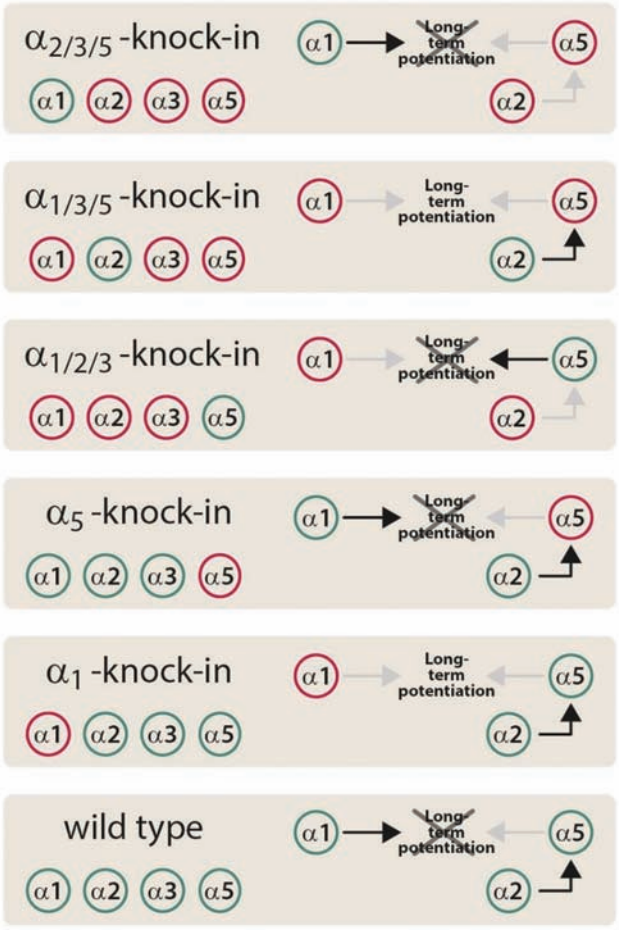

Fig. 7. Minimal schematic model explaining the effects of midazolam on hippocampal long-term potentiation in six different genotypes resulting in our hypothesis. The crucial evidence for our proposed model is marked by a *red box*. Our model assumes that midazolam can inhibit long-term potentiation *via* α_1- and α_5-receptors but not *via* α_2-receptors. α_3-Receptors are only expressed at very low levels and not included in the model. Furthermore, the model assumes an inhibitory action of α_2-receptors onto α_5-receptors as indicated by *arrows*. *Red circles* mark receptor subtypes that are resistant to midazolam, and *green circles* denote responsiveness to the drug. In wild-type mice, long-term potentiation inhibition is mainly produced *via* α_1-receptors, because the activity of α_5-receptors is downregulated by α_2-receptors.

Content 1, table S1, http://links.lww.com/ALN/C845) and legend, a free concentration of midazolam of approximately 10 to 23 nM can be estimated. This concentration range, which is assumed to cause amnesia and moderate sedation in human subjects, is in good accordance with our observation that midazolam was effective in depressing long-term potentiation in hippocampal slices when applied at a concentration of 10 nM.

In clinical anesthesia, midazolam is used because of its profound sedative and hypnotic properties. Plasma concentrations of midazolam causing hypnosis in human subjects are in the range of 300 to 400 ng/ml.[37,38] Persson *et al.*[37] reported that immediately after patients regain consciousness, this concentration is about 166 ng/ml. Based on these data, we assume that brain concentrations of midazolam producing deep sedation should range between 50 and greater than 150 nM (Supplemental Digital Content 1, table S1, http://links.lww.com/ALN/C845). For elucidating the potential contribution of α_1-$GABA_A$ receptors, we quantified the effects of 50 and 500 nM midazolam on $GABA_A$ receptor–mediated synaptic currents in neocortical neurons. For evaluating the specific role of α_1-$GABA_A$ receptors, these studies were conducted in tissue slices derived from wild-type and $\alpha_{2/3/5}$–triple–knock-in mice (only α_1-receptors are sensitive to midazolam). In the wild type, 50 and 500 nM midazolam increased the charge transfer of inhibitory postsynaptic currents to 175 and 240% of control values, respectively. It is interesting to relate these numbers to the effects of etomidate and propofol, two hypnotic drugs that act predominantly *via* $GABA_A$ receptors and produce loss of consciousness at brain concentrations close to 1 μM.[39] In a previous study, we observed that these agents increased charge transfer by 210% (1 μM etomidate) and 220% (1 μM propofol),[26] which is well within the effects produced by 50 and 500 nM midazolam. As a rule of thumb, it seems that loss of consciousness is induced by GABAergic drugs at concentrations that double charge transfer of fast synaptic inhibition.

Zeller *et al.*[8] showed that the sedative properties of benzodiazepines are mediated by α_1-containing $GABA_A$ receptors expressed in forebrain glutamatergic neurons. These receptors constitute the most prominent $GABA_A$ receptor subtype in the cerebral cortex. It has been estimated that about 60% of all $GABA_A$ receptors harbor α_1-subunits.[1] Because of their frequent occurrence, α_1-$GABA_A$ receptors are a prime candidate in mediating the amnestic, sedative, and hypnotic properties of midazolam. We found that clinically relevant concentrations of midazolam significantly enhanced GABAergic synaptic currents in neocortical slices derived from $\alpha_{2/3/5}$–triple–knock-in mice, in which the drug exclusively modulates α_1-$GABA_A$ receptors. At a concentration of 50 nM, midazolam enhanced the charge transferred per average synaptic event in slices from wild-type and $\alpha_{2/3/5}$–knock-in mice by about the same amount (fig. 6A1), suggesting that midazolam's action is predominantly mediated by α_1-receptors at this concentration. However, increasing the drug's concentration from 50 to 500 nM further enhanced the charge transferred per average synaptic event in tissue slices derived from wild-type mice but not in tissue slices derived from $\alpha_{2/3/5}$–knock-in mice. This result suggests that 50 nM is a saturating concentration on α_1-$GABA_A$ receptors but not on non–α_1-$GABA_A$ receptors. It is important to note that this conclusion is based on several assumptions, including similar expression levels of $GABA_A$ receptor subunits in wild-type and knock-in animals, similar expression patterns in different types of neurons, and a selective molecular action of midazolam on classical benzodiazepine-sensitive receptors. Nevertheless, these observations compare well to previous findings. At a concentration of 5 nM, midazolam significantly attenuated high-frequency action potential firing in neocortical slices derived from wild-type, but not in slices prepared from α_1–single–knock-in mice (in this genotype, the drug acts *via* α_2-, α_3-, and α_5-receptors), confirming our previous studies, showing that high-frequency firing is under the control of α_1-receptors.[19,22,40] However, after increasing the concentration of midazolam from 5 to 100 nM, the drug significantly reduced high-frequency firing also in slices derived from α_1–single–knock-in mice, indicating the involvement of non–α_1-receptors at higher (greater than or equal to 100 nM) midazolam concentrations.

Taken together, our findings suggest that an already sedative, low nanomolar concentration of midazolam blocks long-term potentiation *via* α_1-$GABA_A$ receptors. The drug's high potency and efficacy in blocking long-term potentiation prompts the question of how long these effects persist in patients after the treatment. Two studies investigated midazolam's hangover effect about 12 h after subjects received an oral dose of 15 mg.[41,42] The authors report that at a free plasma concentration of about 20 nM, corresponding to a free concentration on brain $GABA_A$ receptors of about 3 nM, midazolam caused low performance in visual memory and in telephone testing tasks. Interestingly, impaired memory was midazolam's only residual effect 10 h after drug intake. If midazolam is used as premedication and for inducing anesthesia and if a plasma half-time of about 2 h is assumed,[43] it seems possible if not likely that the drug is still effective in enhancing the function of α_1-receptors and decreasing cognitive performance, even more than 12 h after terminating midazolam treatment. Moreover, modulation of α_1-receptors may contribute to the occasionally reported phenomenon of transient global postoperative amnesia after midazolam administration.[44–46] The possible involvement of α_1-receptors is consistent with the observation that this complication can be antagonized by flumazenil.

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Competing Interests

Dr. Zeilhofer has a financial relationship with Engrail Therapeutics (San Diego, California), Eliem Therapeutics (Redmond, Washington), and the Swiss National Science Foundation (Bern, Switzerland). Dr. Rudolph has a financial relationship with Elsevier (New York, New York), Concert Pharmaceuticals, Inc. (Lexington, Massachusetts), and the National Institutes of Health (Bethesda, Maryland). The other authors declare no competing interests.

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ANESTHESIOLOGY

Basal Infusion *versus* Automated Boluses and a Delayed Start Timer for "Continuous" Sciatic Nerve Blocks after Ambulatory Foot and Ankle Surgery: A Randomized Clinical Trial

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Perineural catheters with continuous anesthetic infusions are commonly used to provide postoperative analgesia
- Delivering anesthetic by bolus may provide equivalent analgesia with a longer period of effectiveness before depletion of the anesthetic reservoir

What This Article Tells Us That Is New

- Patients undergoing foot or ankle surgery received popliteal–sciatic catheter-reservoir systems delivering ropivacaine by continuous infusion or by a bolus of anesthetic every 2 h
- Those patients receiving bolus anesthetic experienced better pain control and effects of longer duration than those receiving continuous infusions

ABSTRACT

Background: The common technique using a basal infusion for an ambulatory continuous peripheral nerve blocks frequently results in exhaustion of the local anesthetic reservoir before resolution of surgical pain. This study was designed to improve and prolong analgesia by delaying initiation using an integrated timer and delivering a lower hourly volume of local anesthetic as automated boluses. The hypothesis was that compared with a traditional continuous infusion, ropivacaine administered with automated boluses at a lower dose and 5-h delay would (1) provide at least noninferior analgesia (difference in average pain no greater than 1.7 points) while both techniques were functioning (average pain score day after surgery) and (2) result in a longer duration (dual primary outcomes).

Methods: Participants (n = 70) undergoing foot or ankle surgery with a popliteal–sciatic catheter received an injection of ropivacaine 0.5% with epinephrine (20 ml) and then were randomized to receive ropivacaine (0.2%) either as continuous infusion (6 ml/h) initiated before discharge or as automated boluses (8 ml every 2 h) initiated 5 h after discharge using a timer. Both groups could self-deliver supplemental boluses (4 ml, lockout 30 min); participants and outcome assessors were blinded to randomization. All randomized participants were included in the data analysis.

Results: The day after surgery, participants with automated boluses had a median [interquartile range] pain score of 0.0 [0.0 to 3.0] *versus* 3.0 [1.8 to 4.8] for the continuous infusion group, with an odds ratio of 3.1 (95% CI, 1.23 to 7.84; $P = 0.033$) adjusting for body mass index. Reservoir exhaustion in the automated boluses group occurred after a median [interquartile range] of 119 h [109 to 125] *versus* 74 h [57 to 80] for the continuous infusion group (difference of 47 h; 95% CI, 38 to 55; $P < 0.001$ adjusting for body mass index).

Conclusions: For popliteal–sciatic catheters, replacing a continuous infusion initiated before discharge with automated boluses and a start-delay timer resulted in better analgesia and longer infusion duration.

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An ambulatory continuous popliteal–sciatic nerve block can provide potent analgesia at home after foot and ankle surgery.[1] Until recently, there were primarily two local anesthetic delivery modalities: a continuous basal infusion and patient-controlled bolus doses. While providing exclusively patient-controlled boluses can decrease local anesthetic consumption, it also frequently results in inferior analgesia and

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increased opioid consumption.[2] Therefore, during the past 2 decades, the predominant delivery technique has been a continuous basal infusion, frequently combined with patient-controlled bolus doses.[3] Due to weight and bulk limitations, the maximum reservoir volume is about 500 ml, with very few exceptions reported.[3,4] Therefore, the maximum duration of local anesthetic administration at a continuous rate of 6 ml/h is 83 h, although it is more typically 48 to 60 h due to patients' self-administering bolus doses for breakthrough pain.[2] Unfortunately, the pain after many foot and ankle procedures frequently extends beyond this time period.[5] Therefore, decreasing hourly consumption could extend the duration of local anesthetic administration and possibly analgesia.

Portable infusion pump technology has continued to advance, with some devices now capable of delivering bolus doses automatically at a programmed interval.[6] To date, nearly all trials involving ultrasound-guided catheter insertion adjacent to various peripheral nerves have failed to detect analgesic superiority of one administration modality over the other with equivalent hourly local anesthetic volume and dose, especially with the addition of patient-controlled boluses.[6–20] While this suggests that switching from basal to automated boluses is unwarranted,[21] if the latter allows for a decrease in hourly anesthetic consumption while providing noninferior analgesia, it could prolong anesthetic administration and therefore analgesia. For outpatients with a fixed-volume reservoir, automated intermittent boluses offer two different opportunities to improve postoperative analgesia: (1) in the few initial postoperative days and (2) after the time when a basal infusion would have exhausted the anesthetic reservoir.

Another novel feature of some new ambulatory infusion pumps is a start delay timer that allows the pump to be initialized by healthcare providers before discharge but the local anesthetic administration begun only after a programmed number of hours.[22] This function allows conservation of the local anesthetic reservoir in the hours after discharge while a preoperative single-injection peripheral nerve block remains effective.

Materials and Methods

This parallel group study adhered to Good Clinical Practice quality standards and ethical guidelines defined by the Declaration of Helsinki. Study protocol approval, as well as data and safety oversight, were conducted by the University of California San Diego Institutional Review Board (approval No. 200247). Written informed consent was obtained from all participants. The trial was prospectively registered at clinicaltrials.gov (NCT04458467, Principal Investigator: Brian Ilfeld, date of registration: July 7, 2020) before initiation of enrollment, and the trial protocol is available at http://clinicaltrials.gov. The trial was conducted in accordance with the original protocol.

Enrollment was offered to adults (18 yr and older) who were scheduled to undergo unilateral ambulatory foot and/or ankle surgery associated with pain that the surgeon expected to persist beyond the duration of a single-injection nerve block and require treatment with oral opioid analgesics. Patients eligible for the study were called the night before surgery by an investigator to offer enrollment. If not available by telephone, patients were offered enrollment in person before surgery only if there was sufficient time to fully discuss the study, answer all questions, read the consent form thoroughly, and give patients time to make an informed decision. All participants were enrolled at one of two hospitals or an ambulatory surgery center, all of which are part of a single academic institution in Southern California. Patients were excluded if they had clinically apparent neuropathy of the ipsilateral sciatic nerve and its branches and/or innervating muscles; current daily opioid use within the previous 4 weeks; body mass index greater than 35 kg/m^2; surgery outside of ipsilateral sciatic and saphenous nerve distributions (*e.g.*, iliac crest bone graft); pregnancy; or incarceration.

Preoperative Procedures

After applying standard monitors, providing oxygen by face mask, and positioning the patient in the prone position, intravenous midazolam and fentanyl were titrated for patient comfort while ensuring that the patient remained responsive to verbal cues. A 13- to 6-MHz, 38-mm linear array ultrasound transducer (Edge II, SonoSite, USA) was used to visualize the sciatic nerve proximal to the bifurcation with a short-axis view. The catheter site was sterilely prepped and draped, and the needle entry site and underlying muscle were anesthetized with 1% lidocaine (3 ml).

Perineural Catheter Insertion

A 17-guage Tuohy needle (FlexTip Plus, Teleflex Medical, USA) was inserted on the posterolateral aspect of the leg, from lateral to medial, using an in-plane, short-axis, ultrasound-guided technique, and 1 to 2 ml of normal saline was injected to open the space adjacent to the nerve. A 19-gauge flexible, single-orifice perineural catheter was inserted under ultrasound guidance 2 to 3 cm beyond the needle tip, and the needle was removed over the catheter. Ropivacaine (0.5%) with 5 to 10 μg/ml epinephrine (20 ml) was injected in divided doses through the

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catheter under ultrasound visualization. The catheter was then secured with clear, occlusive dressings. To facilitate tourniquet placement in the operating room, the catheter was taped up the lateral thigh.

A successful block was defined as sensory (decreased temperature discrimination to ice) and motor (decreased dorsi- and plantar-flexion strength) in the tibial and peroneal nerve distributions within 15 min of injection (unless the foot was not accessible due to a splint) as compared to the contralateral foot. If the planned surgical procedure was anticipated to produce pain in the saphenous nerve distribution, a single-injection saphenous nerve block was performed with ropivacaine (0.5%) with 5 to 10 μg/ml epinephrine (10 to 20 ml).

Treatment Group Allocation

After confirming a successful block in the sciatic distribution, participants were randomized using a computer-generated list (prepared by the University of California San Diego Investigational Drug Service) and provided to the investigators in opaque, sealed, sequentially numbered security envelopes to one of two treatment groups (1:1 ratio) in blocks of four: (1) automated bolus with a 5-h delayed start or (2) continuous infusion with an immediate start. These envelopes were opened by a member of the care team (*e.g.*, block nurse or regional anesthesiology fellow) who was not a coinvestigator and not involved in study data collection. Thus, both the participants and investigators were blinded to treatment group.

Intraoperative Procedures

Patients received either intravenous propofol sedation or general anesthesia consisting of inhaled volatile anesthetic with or without nitrous oxide in oxygen. Opioids were administered at the discretion of the intraoperative anesthesia team.

Postoperative Procedures

After completion of the surgical procedure, an electronic infusion pump (Nimbus II PainPRO, InfuTronix, USA) with a 500-ml reservoir of 0.2% ropivacaine was attached to the perineural catheter. The pump was programmed based on the participant's randomization group by an investigator who was not involved in data collection or analysis (table 1). One group received continuous infusion with no delay: the ropivacaine infusion was started immediately on attachment of the pump (6 ml/h basal infusion, 4-ml patient-controlled bolus, 30-min lockout). The other group received automated boluses with a 5-h delay: the infusion pump entered a pause mode after attachment of the pump to delay administration of local anesthetic for 5 h. The pause could be overridden by the participant if the initial peripheral nerve block resolved sooner than anticipated. After the 5-h delay, or when the patient overrode the delay, local anesthetic administration was initiated (automated 8-ml boluses every 2 h, 4-ml patient-controlled bolus with a 30-min lockout).

Participants were discharged with a prescription for the synthetic opioid oxycodone (5-mg tablets) for supplementary analgesia and contacted by telephone daily for 6 days after surgery to collect study outcome measures. Upon ropivacaine reservoir exhaustion, participants or their caretakers were instructed by phone on removing the perineural catheter.

Outcome Measures

We hypothesized that, compared with a continuous basal infusion initiated before discharge, perineural local anesthetic administered with automated boluses at a lower dose and a 5-h delay after discharge would (1) provide at least noninferior analgesia during the period that both techniques are functioning (primary outcome: average pain score the day after surgery) and (2) result in a longer duration of administration (primary outcome: hours from initiation until reservoir exhaustion) for popliteal–sciatic catheters after ambulatory foot and ankle surgery. We utilized dual primary endpoints with a serial testing strategy, such that superiority for overall duration of local anesthetic infusion would be tested only if the average pain scores on the first postoperative day were found to be at least noninferior.

The participants and investigators collecting the data were masked to treatment group assignment. The first four items of the Brief Pain Inventory were collected daily[23]: worst, average, least, and current surgical pain measured using a numeric rating scale. Additional outcomes included daily opioid consumption, number of sleep disturbances due to pain, degree of sensory block (measured on a 0 to 10 scale, where 0 indicates no deficits, and 10 indicates completely insensate), and satisfaction with postoperative analgesia (where 0 indicates very dissatisfied, and 10 indicates completely satisfied). In addition, for medical purposes, we collected the following information: local anesthetic leakage (binary) and the cause for discontinuation of the infusion (*e.g.*, completion of infusion, accidental dislodgement).

Statistical Methods

The study was powered for two primary endpoints: (1) the average numeric rating scale queried on postoperative day 1 and (2) the duration of treatment from when the infusion pump was initiated until local anesthetic reservoir exhaustion. The dual hypotheses were tested with a serial testing strategy, such that hypothesis 2 would not be formally tested unless the conclusion of hypothesis 1 was at least "noninferior." Following the approach described by Althunian *et al.*,[24] noninferiority was assessed by comparing the lower limit of the two-sided 95% CI for the difference (CB minus AB) on the numeric rating scale (range, 0 to 10) to a prespecified noninferiority margin of 1.7 numeric rating scale units (fig. 1, A and B). This provided evidence that

Table 1. Perineural Ropivacaine Administration Profile by Treatment Group

Treatment Group	Basal Infusion		Automated Boluses		Total, Average/h	
	ml/h	mg/h	ml/2 h	mg/2 h	ml/h	mg/h
Basal infusion	6	12	0	0	6	12
Automated boluses	0	0	8	16	4	8

Only the scheduled/obligatory local anesthetic is included in the table, but both treatment groups also had a 4-ml patient-controlled bolus available every 30 min.

the analgesia provided by the novel automated boluses was no worse than 1.7 numeric rating scale units compared to a basal infusion. The same two-sided 95% CI can be compared to 0.0 numeric rating scale to conclude superiority of automated boluses at the 5% significance level.

We assessed the balance of randomized groups on baseline and procedural characteristics using absolute standardized difference, defined as the absolute difference in means, mean ranks, or proportions divided by the pooled SD. Baseline variables with absolute standardized difference greater than 0.47 (*i.e.*, $1.96 \times\sqrt{(1/n1 + 1/n2)}$) were noted and included in a linear regression model to obtain an estimate of the treatment group differences adjusted for the imbalanced covariate(s).[25,26] If residuals from the linear regression indicated violations of key assumptions (*i.e.*, homoscedasticity or Gaussian distribution), data transformations and/or alternative generalized linear models were applied, as appropriate.

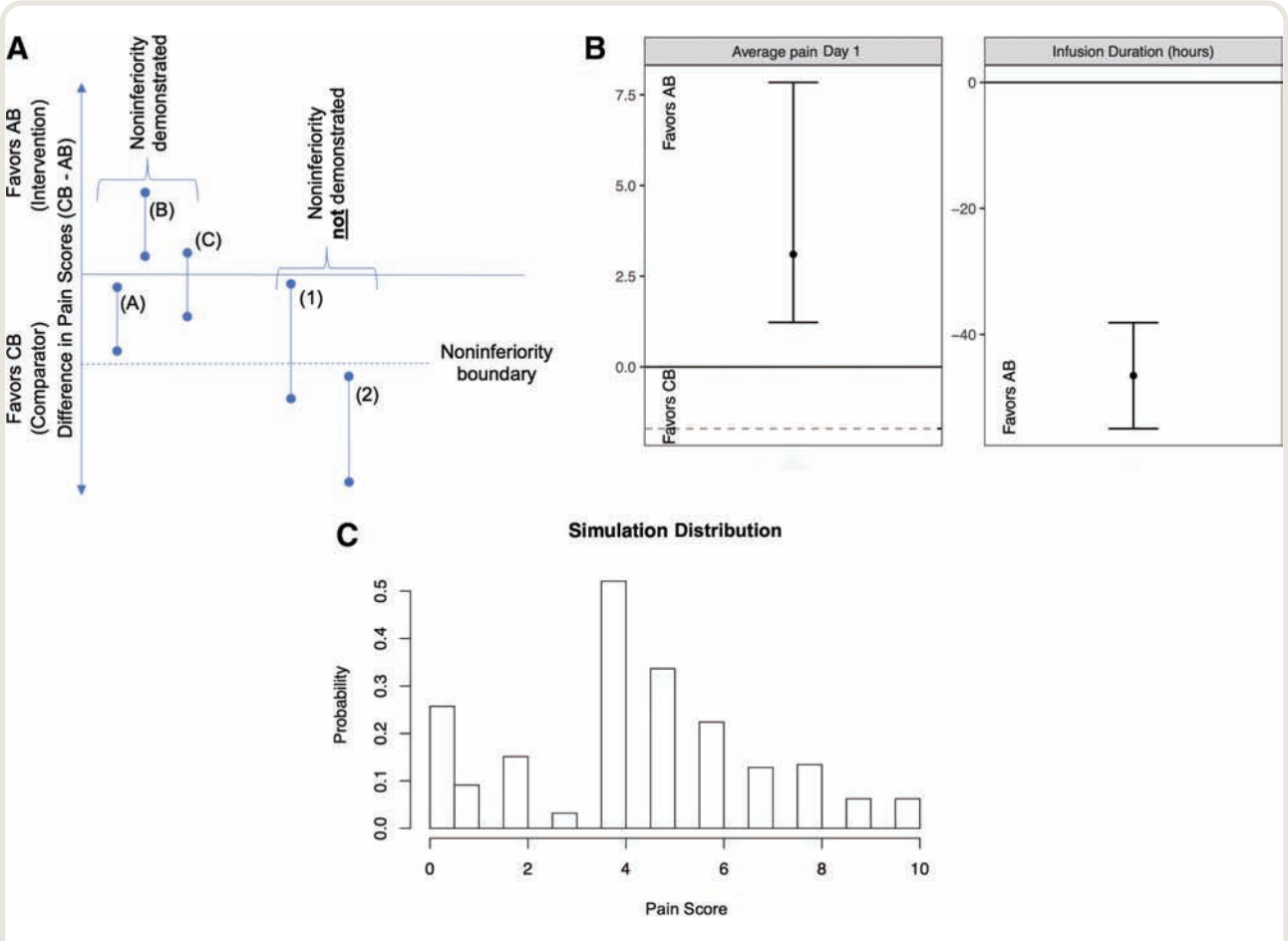

Fig. 1. (*A*) Noninferiority margin and framework for concluding noninferiority or not. (*B*) CI for primary models adjusting for body mass index. The infusion duration is modeled with a linear regression model, and the average pain is modeled with a proportional odds logistic regression model. (*Left*) Noninferiority margin shown as a dashed line at –1.7 units on a numeric rating scale. (*C*) Discrete distribution used for simulations based on the distribution observed in the above bifurcation group in the work of Monahan *et al.*[29]

Primary outcomes were analyzed using an ordinal regression model for continuous scales to compare "average" pain scores on the first postoperative day (hypothesis 1) and a linear regression model for infusion duration (hypothesis 2).[27,28] Secondary outcomes were analyzed by Wilcoxon–Mann–Whitney test or linear models as appropriate with covariates for any imbalanced covariates. No multiplicity adjustments were applied for these analyses. All analyses were conducted using R version 4.1.1. Treatment group allocation was only revealed to investigators after statistical analysis.

Sample Size and Power Estimation

Separate power analyses were conducted for both hypotheses, with hypothesis 2 contingent on hypothesis 1.

Hypothesis 1. Power was simulated based on the distribution of the numeric rating scale observed in previously published studies of pain scores after foot and ankle surgery with a continuous popliteal–sciatic infusion.[29] Specifically, numeric rating scale scores were simulated from a discrete distribution that generated an expected interquartile range from 1 to 4, and a median of 3 numeric rating scale units. One thousand trials were simulated in which two groups, n = 35 per group, were assumed to follow the same discrete distribution (fig. 1C). Each trial was submitted to a Wilcoxon–Mann–Whitney test, and 95% CIs were derived.[27] Of the 1,000 trials, 792 (79.2%) correctly resulted in a conclusion of noninferiority, suggesting that the probability that the trial correctly concludes noninferiority is about 80% when the groups follow exactly equivalent distributions.

Hypothesis 2. If the test for hypothesis 1 concluded noninferiority or superiority, the difference in overall duration of administration would be tested using the Wilcoxon–Mann–Whitney test. Power was approximated by an independent sample *t* test calculation. Assuming a SD of 37 h (corresponding to an interquartile range of 50 to 100 h), a sample size of n = 35 would provide 80% power to detect a mean group difference of 25 h with a two-sided α of 5%.

Results

A total of 71 participants were enrolled beginning July 15, 2020, and ending March 10, 2021 (fig. 2). Enrollment was ceased when the target sample size had been obtained, and data collection was finished on March 16, 2021. All but one participant had a successful sciatic nerve block. The remaining 70 participants were randomized and equally divided between the treatment groups, and all randomized participants were included in primary and secondary analyses per the intention-to-treat principle. All participants remained blinded to treatment group for the duration of follow-up. Outcome assessors remained blinded to treatment group allocation until after the statistical analysis was complete. Participants were summarized and compared on potentially confounding baseline and procedural characteristics (table 2). All factors were balanced between the two groups with the exception of body mass index, which was potentially imbalanced between groups with a higher body mass index in the continuous infusion group (absolute standardized difference, 0.693). Therefore, the primary analysis was adjusted for body mass index. A unit increase in body mass index had little effect on the odds of worse pain (odds ratio, 1.0; 95% CI, 0.9 to 1.1). However, it did have an effect on infusion duration such that larger body mass index was associated with shorter infusion duration (–1.1 h per body mass index increase; 95% CI, –2.0 to –0.3; $P = 0.008$). In a model controlling for the independent effects of height and weight, the continuous infusion group had a shorter mean infusion duration (–46.1 h; 95% CI, –54.6 to –37.5; $P < 0.001$), higher weight was associated with shorter infusion duration (–0.4 h/kg; 95% CI, –0.7 to –0.1; $P = 0.007$), and taller heights were associated with longer infusion duration (54.0 h/m; 95% CI, 3.9 to 104.2; $P = 0.035$). The adjusted analyses of all secondary outcomes were consistent with the unadjusted analyses. There were no missing data for any baseline variables or primary outcomes. Four subjects in the automated bolus group could not be reached by telephone for one of the nonprimary outcome telephone calls *versus* one subject in the continuous infusion group. Thus, secondary outcome data were incomplete for these days. No data were lost or excluded.

Primary Outcomes

The day after surgery, participants with automated boluses had a median [interquartile range] pain score of 0.0 [0.0 to 3.0] *versus* 3.0 [1.8 to 4.8] for the continuous infusion group (unadjusted 95% CI, –2.0 to 0.0; $P = 0.007$; fig. 3A). The odds of worse average pain on day 1 with continuous basal infusion, adjusting for body mass index, were 3.1 (95% CI, 1.2 to 7.8; $P = 0.033$). Local anesthetic reservoir exhaustion in patients with automated boluses occurred at a median [interquartile range] of 119 h [109 to 125] *versus* 74 h [57 to 80] for the continuous infusion group (difference, 47; 95% CI, 42 to 53; $P < 0.001$; fig. 3, B and C). Adjusting for body mass index, the difference was also 47 h (95% CI, 38 to 55; $P < 0.001$).

Secondary Outcomes

Daily average and worst pain scores were lower for the bolus *versus* basal groups at nearly all time points through postoperative day 5 (fig. 4, A and B). Automated boluses reduced the median cumulative opioid consumption by 83%, from 9.0 mg [3.5 to 13.0] to 1.5 mg [0.5 to 5.0] ($P < 0.001$; fig. 4C) and reduced cumulative sleep disturbances by 75%, from 4.0 [2.0 to 10.0] to 1.0 [0.0 to 3.0] ($P < 0.001$; table 3). Participants receiving automated boluses experienced more numbness at all time points (table 3), although local anesthetic leakage did not differ

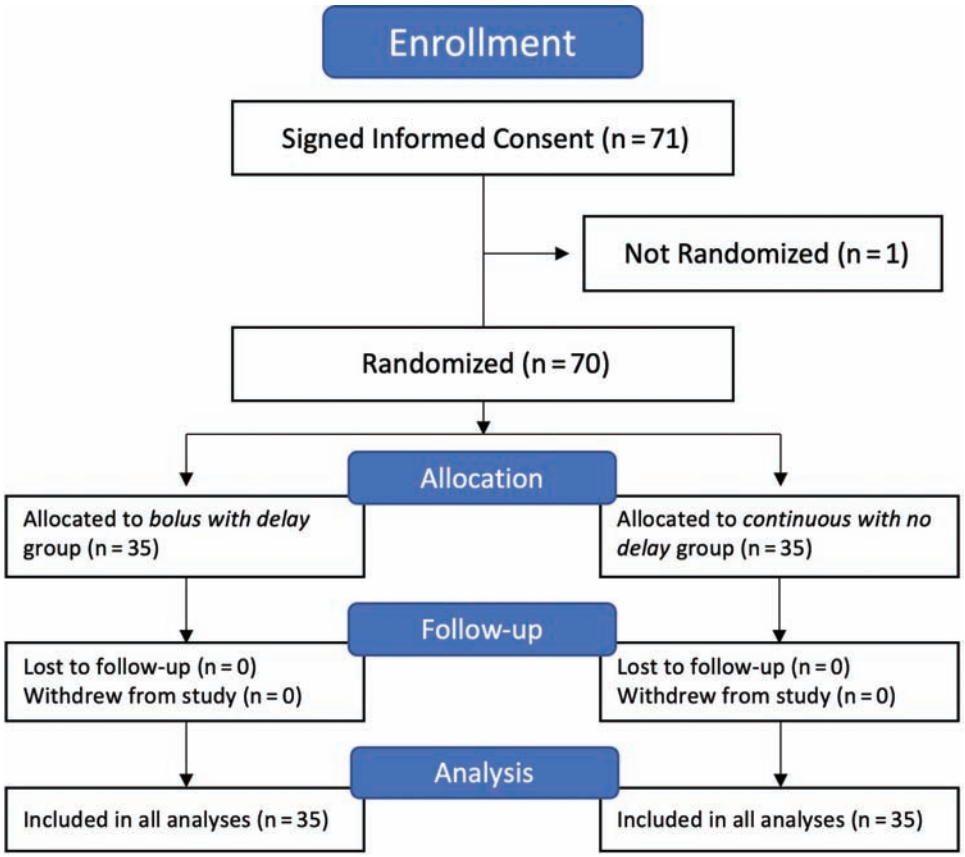

Fig. 2. Consolidated Standards of Reporting Trials (CONSORT) diagram demonstrating the flow of participants through the study. One participant was not randomized due to block failure.

Table 2. Anthropometric and Surgical Information

Factor	Bolus (N = 35)	Continuous (N = 35)	Standard Differences*
Age, yr	48 ± 17	52 ± 18	0.257
Female, n (%)	19 (54)	22 (63)	0.175
Body mass index, kg/m^2	26.7 ± 4.5	30.0 ± 5.2	0.693
Weight, kg	81.5 ± 19.7	87.1 ± 17.3	0.304
Saphenous blocked, n (%)	33 (94)	30 (86)	0.289

The values are reported as means ± SD or percentages of treatment group, as appropriate.

*Absolute standardized differences: the absolute difference in means mean ranks, or proportions divided by the pooled SD, with a criteria absolute standardized difference of 0.46 or greater considered as imbalanced $\left(1.96\times\sqrt{\frac{(n1+n2)}{n1\times n2}}=0.46\right)$.

between treatments (table 3), and satisfaction with analgesia differed only on postoperative days 1 and 4 (table 3).

Adverse Events and Protocol Deviations

Four participants in the automated bolus group and six in the continuous infusion group accidentally dislodged their perineural catheters before infusion completion. All participants whose catheters accidentally dislodged were offered catheter replacement; however, only one participant elected to have the catheter replaced.

One participant in the automated bolus group required revision surgery due to a surgical complication on postoperative day 4 from the initial surgery. This participant's catheter had accidentally dislodged on the third postoperative day. The anesthesia provider caring for this participant on the day of the second surgery placed a new popliteal–sciatic perineural catheter with our institution's conventional settings (continuous infusion at 6 ml/h with 4-ml patient-controlled bolus and 30-min lockout) without knowing the participant was part of a randomized trial. After surgery, the decision was made to continue with these settings while the participant remained an inpatient to provide our institution's normal standard of care. The participant was retained within his randomized group per the intent-to-treat principle. No falls, catheter-related infections, nerve injuries, or other treatment-emergent complications were observed in either group.

Discussion

The main findings of this study are that, compared with a continuous basal infusion initiated before discharge *via* a popliteal–sciatic catheter, perineural local anesthetic administered with automated bolus doses at a lower volume/dose and a 5-h delay after discharge resulted in (1) superior analgesia during the period that both modalities were functioning and (2) a longer duration of anesthetic administration. Significant benefits were identified for pain scores, opioid consumption, and sleep quality for nearly all of the first 5 postoperative days, when both techniques administered local anesthetic, as well as after the reservoir exhaustion of the basal infusion participants. Our study is unique relative to nearly all previous investigations comparing automated boluses to a basal infusion in that (1) we included an integrated start-delay timer; (2) we did not define a maximum treatment period but rather removed catheters only after reservoir exhaustion; (3) we collected data 6 days after surgery, which is two to three times longer than previous investigations; and (4) we decreased mandatory average hourly local anesthetic delivery, while most others compared equivalent volumes/doses.[6–20,30–32]

Basal *versus* Automatic Boluses

For popliteal–sciatic catheters after foot and ankle surgery, previous studies suggest that providing a basal infusion maximizes analgesia and other benefits compared with exclusively patient-controlled boluses, presumably due to the observed decrease in anesthetic volume/dose administered when patients must trigger the boluses themselves.[2,33] However, based on findings for epidural catheters,[34–36] it was theorized that increasing the volume of local anesthetic introduced at a single time point—a bolus—might improve perineural spread compared with an equivalent volume/dose provided as a basal infusion providing superior analgesia.[21] Indeed, the first investigation replacing patient-controlled with automated boluses for sciatic catheters found an analgesic benefit over an equivalent volume/dose administered with a basal infusion.[6] However, by adding patient-controlled bolus doses to these two regimens, the difference in pain scores disappeared.[7]

Our study was somewhat unique in that we intentionally decreased the average volume/dose of local anesthetic administered with automated boluses (8 ml every 2 h) by 33% compared with the basal infusion (12 ml every 2 h). We had hoped to provide noninferior analgesia and conserve local anesthetic, thereby prolonging overall administration and, consequently, analgesia. However, we did not anticipate our finding of superior analgesic effects with the automated boluses while both treatment groups were receiving local anesthetic. Our findings are in contrast to multiple other investigations involving perineural sciatic catheters.[7–9] Taboada *et al.*[6,7] identified no analgesic or opioid-sparing benefits comparing a 5-ml automated hourly bolus with a 5-ml/h basal infusion, both with available patient-controlled boluses. The difference may be due to our larger bolus volume—8 *versus* 5 ml—even though it was administered only every 2—*versus* 1—h, thus providing a lower average hourly volume/dose.

However, two additional randomized trials that utilized larger (9.8 to 10 ml) automated boluses every 2 h *via* popliteal–sciatic catheters—a larger volume than our study—identified no analgesic benefits when compared with a basal infusion of the same average hourly volume/dose (5 ml/h), both with available patient-controlled boluses.[8,9] While their results may appear to contradict our findings, both studies did identify other differences between treatments. Short *et al.*[8] reported increased motor block in the automated bolus group, suggesting that bolus doses even in an equivalent volume/dose with a basal infusion have different effects. While the sensory block did not reach statistical significance after correction for multiple comparisons, the use of a 3-point (*vs.* our 10-point) scale may have provided insufficient dispersion given their sample size.

Similarly, while Breebaart *et al.*[9] failed to identify analgesic benefits, their participants receiving automated boluses consumed a smaller volume of local anesthetic, again suggesting a difference between treatments. In contrast, patients receiving automated boluses experienced a 64% incidence of "numbness"—similar to our findings—identical to their basal group. Failing to detect a difference in sensory block between their treatments may be due to their use of binary response (present/absent), while our 0 to 10 sensory block scale may have provided more sensitivity to differences between treatments.

Our analgesic-related results may differ from those of Short *et al.*[8] and Breebaart *et al.*[9] because of seemingly insignificant protocol variations, such as differing catheter insertion approaches (in- *vs.* out-of-plane needle advancement), catheter tip locations (subparaneural *vs.* supraparaneural), or local anesthetic (0.2% ropivacaine *vs.* 0.125% levobupivacaine). Additionally, participants in the current study had the potential to prolong their local anesthetic infusion—and thus analgesia—by reducing demand bolus use and may have tolerated higher pain scores to prolong the infusion duration and its analgesic effects.

Recent findings of analgesic improvement with automated boluses *versus* a basal infusion for paravertebral perineural catheters add credence to our results.[30] This topic will require additional research to answer the multitude of questions raised by the current study.

Administration Duration

Although our study protocol ensured less mandatory local anesthetic delivery in the automated bolus group compared with participants with a basal infusion (8 ml *vs.* 12 ml every 2 h), we provided both treatment groups with the ability to self-administer additional boluses every 30 min. We had anticipated that participants with the automated

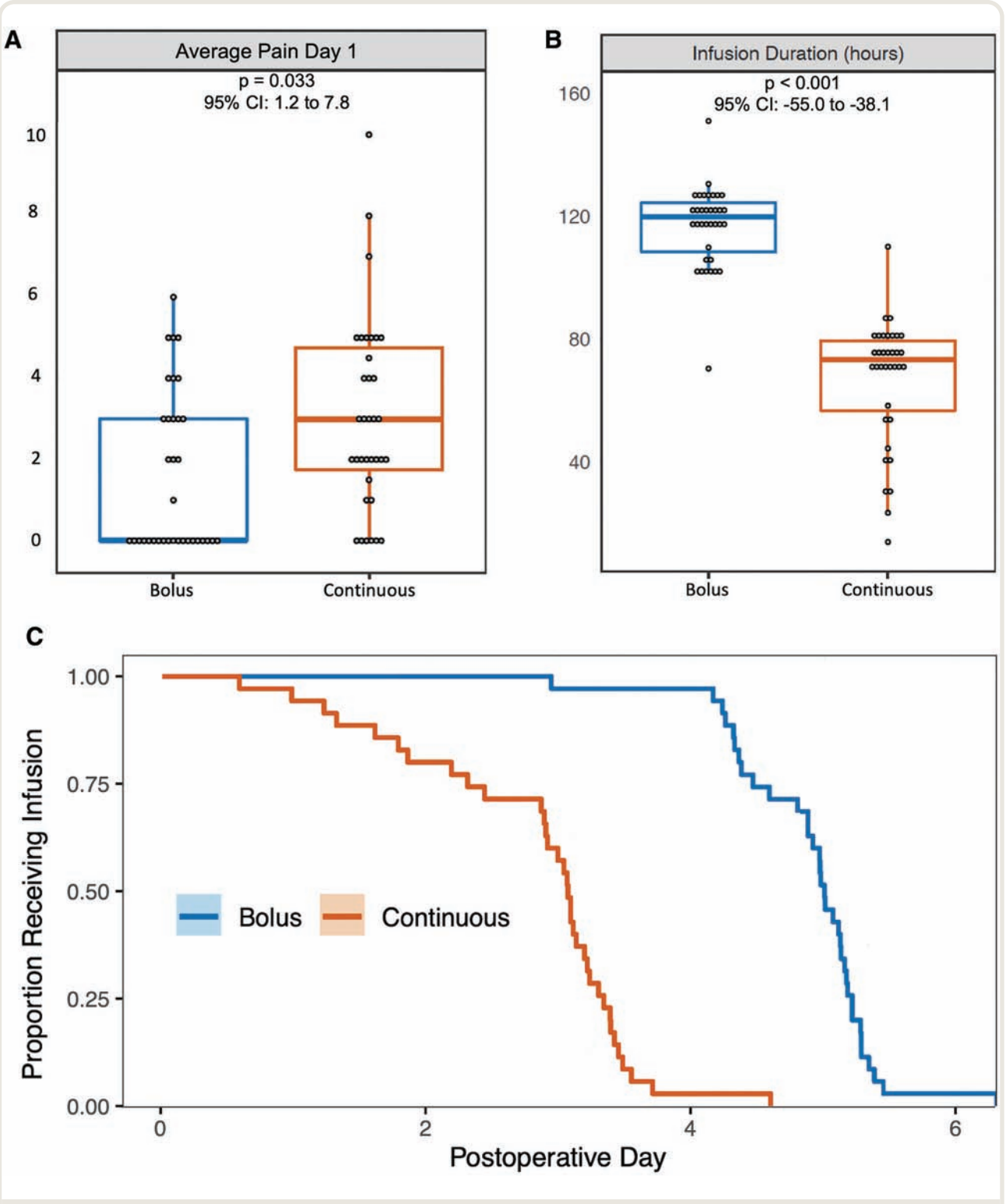

Fig. 3. Effect of delivery modality on average pain scores from postoperative day 1 and duration of local anesthetic administration. Pain severity is indicated using a numeric rating scale (*A*) with 0 equal to no pain and 10 being the worst imaginable pain. Treatment duration is presented in hours (*B*). The data are expressed as individual participants (*circles*) and median (*darkest horizontal bar*) with 25th to 75th percentiles (*box*), and the *whiskers* extend beyond the quartiles by 1.5 times the interquartile range or the extreme value (whichever is closer). In addition, the Kaplan–Meier curve (*C*) demonstrates the percentage of participants whose infusions continued at given time intervals.

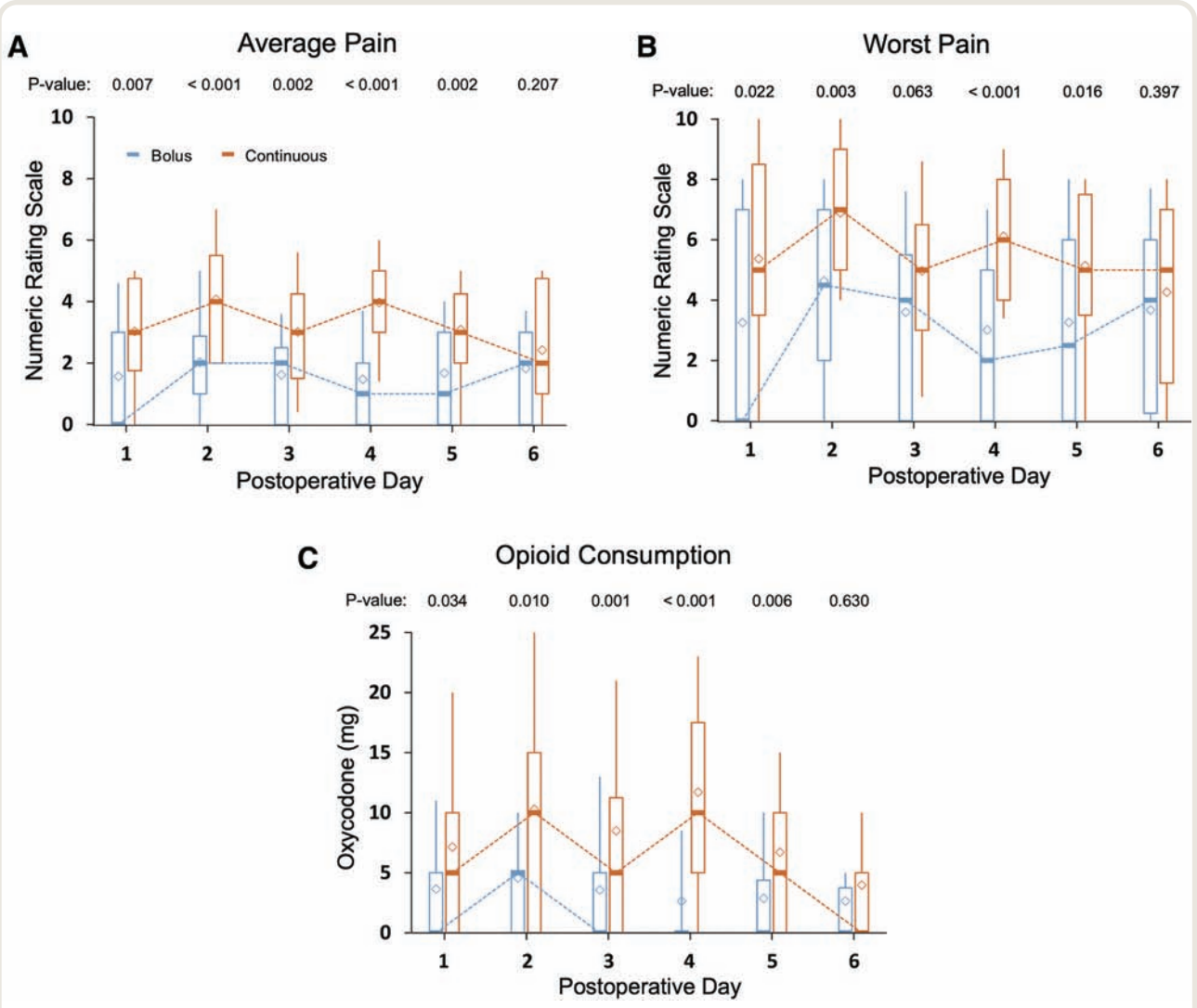

Fig. 4. Effect of delivery modality on daily average pain scores (*A*), worst pain scores (*B*), and opioid consumption (*C*). The data are expressed as median (*darkest horizontal bar*) and mean (*diamond*) with 25th to 75th percentiles (*box*), and the *whiskers* extend to the 10th to 90th percentiles.

boluses would self-administer more boluses to compensate for their scheduled 4-ml local anesthetic deficit every 2 h. However, this did not occur, with automated boluses decreasing local anesthetic consumption and prolonging administration 61% from approximately 3 to 5 days (only 5 h of which was due to the start-delay timer). Previous studies of basal *versus* bolus dosing of popliteal–sciatic catheters concluded both treatments at the same time regardless of residual reservoir volume (usually after 24 to 48 h).[6–9] This permitted the comparison of local anesthetic consumption and relative benefits during the initial 1 to 2 postoperative days but not subsequent consumption or analgesic effects. For inpatients who have access to reservoir refills, the anesthetic-sparing appears to have little importance given the extraordinarily rare occurrence of local anesthetic toxicity during perineural administration.[37] However, for ambulatory patients with a fixed reservoir volume, decreasing local anesthetic consumption allows for an extended administration duration and therefore prolonged analgesia delivery.

Our findings that patients who continued to receive automated bolus doses experienced superior analgesia compared to participants who had previously exhausted their anesthetic reservoir with a basal infusion is unsurprising, yet this has not been previously documented with a prolonged 6-day period of evaluation as in our study. Given our findings of improved analgesia while both administration modalities were delivering local anesthetic, our results are applicable to inpatients as well.

Table 3. Secondary Outcomes Comparing the Bolus *versus* Continuous Infusion Administration Methods

Outcome	Bolus (N = 35)	Continuous (N = 35)	*P* Value
Sleep disturbances			
Postoperative day 1	0 [0, 0.5]	1 [0, 1.5]	0.011
Postoperative day 2	0 [0, 1]	1 [0.5, 2]	< 0.001
Postoperative day 3	0 [0, 0]	0 [0, 1]	0.023
Postoperative day 4	0 [0, 0]	0 [0, 1]	< 0.001
Postoperative day 5	0 [0, 0]	0 [0, 1]	0.017
Postoperative day 6	0 [0, 1]	0 [0, 1]	0.666
Numbness (0 = no numbness, 10 = insensate)			
Postoperative day 1	10 [8, 10]	8 [5, 10]	0.015
Postoperative day 2	8 [7, 10]	5 [3, 8]	< 0.001
Postoperative day 3	8 [5.5, 9.5]	5 [0,8]	0.001
Postoperative day 4	8 [5.3, 9.4]	0 [0, 0]	< 0.001
Postoperative day 5	6 [2.3, 8]	0 [0, 0]	< 0.001
Postoperative day 6	0 [0, 0]	0 [0, 0]	0.003
Satisfaction (0 = no numbness, 10 = insensate)			
Postoperative day 1	10 [9, 10]	9 [8, 10]	0.021
Postoperative day 2	9 [8, 10]	9 [7, 10]	0.087
Postoperative day 3	10 [9, 10]	10 [9, 10]	0.495
Postoperative day 4	10 [9, 10]	9 [7, 10]	0.010
Postoperative day 5	10 [9, 10]	10 [8.5, 10]	0.083
Postoperative day 6	10 [9, 10]	10 [8.6, 10]	0.287
Leakage			
Postoperative day 1	0 [0, 0]	0 [0, 0]	0.967
Postoperative day 2	0 [0, 0]	0 [0, 0]	0.369
Postoperative day 3	0 [0, 0]	0 [0, 0]	0.644
Postoperative day 4	0 [0, 0]	0 [0, 0]	0.860
Postoperative day 5	0 [0, 0]	Not applicable	
Postoperative day 6	0 [0, 0]	Not applicable	

The values are reported as median [interquartile range].

Start-delay Timing

The 5-h start delay was chosen as a conservative estimate to ensure that the first bolus dose occurred before the analgesia provided by the initial preoperative single injection 0.5% ropivacaine block would resolve.[38] Our goal was to prolong the duration of the 0.2% ropivacaine infusion as long as possible while ensuring that the participants did not experience pain with the initial block resolution.[39] To minimize this likelihood, participants could override the delay if they began experiencing pain before the automatic activation of the first bolus. Start-delay timers are a relatively novel function available on few electronic infusion pumps, and the results of the current study suggest that this feature may be used to prolong the total duration of an ambulatory perineural ropivacaine infusion and the analgesia provided.

Anesthetic Consumption

An unanticipated finding was that higher weight was associated with shorter infusion duration, while taller height was associated with longer infusion duration. These results suggest a complex relationship between body morphology and local anesthetic requirements that is previously unreported in the literature. Specifically, these data indicate that obesity increases local anesthetic requirements. As such, patients with a higher body mass index may benefit from automated bolus dosing to an even greater degree than their lower–body-mass-index counterparts. Further research is indicated to elucidate this complex relationship between body habitus and local anesthetic requirements.

Study Limitations

Although the participants of this investigation were masked to treatment group assignment, the audible pump activation occurring either continuously or at 2-h increments may have allowed some participants to deduce their randomization group. Further, although the infusion settings were not obviously displayed on the pump, participants did have access to the pump controls and could potentially have investigated their treatment group. However, it is improbable that the majority of patients had a preconceived preference for one technique over the other. In addition, the continuous activation of the pump could have interfered with sleep more in the continuous groups compared to the automated bolus group, who would only potentially be awoken by the pump activation every other hour. Regarding study design, two interventions were undertaken to increase the duration of the ropivacaine infusion in the experimental group: a start-delay timer and automated boluses with lower basal

dosing. Given the 5-h start delay for the treatment group and median of 45-h overall increase in administration duration for these participants, we assume that the duration difference between treatments would have been 40 h if both groups had been provided with the 5-h start delay. Last, while we used an observer-masked design to minimize outcome assessor bias, an unmasked healthcare provider was required to program the infusion pump, and therefore we could not implement a triple-masked study. However, the unmasked healthcare provider had limited interaction with the participants, and the treatment groups were not identified to the statistician until the analysis was completed.

Conclusions

The results from this study suggest a possible paradigm shift for postoperative perineural catheters, 75 yr after Ansbro[40] described the first continuous peripheral nerve block: replacing a basal infusion with delayed-start large automated boluses to both improve analgesia potency and prolong analgesia delivery. Evidence may be found in one previously published investigation that—like our study and unlike nearly all previous basal *versus* automated bolus trials—administered less mandatory local anesthetic for the automated boluses relative to the basal infusion.[13] Rao Kadam *et al.*[13] randomized 20 subjects with bilateral transversus abdominis plane catheters to receive ropivacaine (0.2%) as either provider-administered boluses of 20 ml every 8 h or a pump-administered basal infusion of 8 ml/h for each catheter. Although the boluses administered only 31% as much volume/dose as the basal infusion (2.5 *vs.* 8.0 ml/h), no differences in pain scores or supplemental opioid consumption were identified during the 48-h study period. This study was possibly underpowered but combined with our own findings suggests that additional research intentionally lowering the average hourly volume/dose for automated boluses may better match the durations of analgesic delivery and surgical pain.[13]

To summarize, for popliteal–sciatic catheters after ambulatory foot and ankle surgery, replacing a continuous infusion initiated before discharge with automated boluses and a start-delay timer resulted in both better analgesia and a longer duration of the infusion. Reservoir exhaustion occurred on approximately the third postoperative day with conventional infusion settings and the fifth postoperative day when automated boluses and a start-delay timer were used. Additional research is required to determine whether these results may be replicated for catheters in other anatomic locations and to optimize the various associated factors such as catheter insertion protocol, automated and patient-controlled bolus volumes and frequency, and local anesthetic type and concentration.

Research Support

InfuTronix (Natick, Massachusetts) provided the electronic pumps used in this study. The company was given the opportunity to review the protocol and suggested minor revisions. The investigators retained full control of the investigation, including study design, protocol implementation, data collection, data analysis, results interpretation, and manuscript preparation.

Competing Interests

Drs. Finneran, Said, Swisher, Gabriel, and Ilfeld and the University of California have received funding and product for other investigations from infusion pump manufacturer InfuTronix; the cryoneurolysis device manufacturer Epimed (Farmers Branch, Texas); and the peripheral nerve stimulation device manufacturer SPR Therapeutics (Cleveland, Ohio). Dr. Gabriel has worked as a paid consultant for Avanos (Alpharetta, California). Dr. Donohue has received support for other research projects from the National Institutes of Health (Bethesda, Maryland), Biogen/Eisai (Cambridge, Massachusetts), and Roche (Basel, Switzerland). The other authors declare no competing interests.

Reproducible Science

Full protocol available at: jfinneran@health.ucsd.edu. Raw data available at: jfinneran@health.ucsd.edu.

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Labeled Surgical Caps: A Tool to Improve Perioperative Communication

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Communication in the operating room is enhanced by knowing everyone's names and roles. This is challenging among different disciplines.[1] Name badges can be small, flipped, and concealed by personal protective equipment. A labeled scrub cap may make name and role more readable from a distance (fig.). Role clarity and addressing individuals by name enhance communication, teamwork, and patient safety.[2] Role clarity may also promote diversity, equity, and inclusion. For example, women were 20% less likely to be thought of as surgeons by patients.[3] In an anesthesiology care team model, role confusion can sometimes be misleading to patients and medical professionals.

At our hospital, each team member received several labeled caps to allow for laundering and a clean cap to be worn in the perioperative area. Although there was previous concern regarding head coverings and infection risk, multiple organizations, including the American Society of Anesthesiologists (Schaumburg, Illinois), The Joint Commission (Oakbrook Terrace, Illinois), and surgical and nursing societies, have concluded that there is no association between surgical cap type and surgical site infection.[4] Each interprofessional group designated its preferred notation. Physicians chose "Doctor" with full name, whereas nurses preferred "RN" with first name.

Individuals have put names on their surgical caps for years. Institutional sponsorship supporting the majority of providers wearing labeled caps represents a culture change enhancing effective team communication. Widespread use of low-cost caps with clearly identifiable names and roles may improve communication, role clarity, provider well-being, and diversity, equity, and inclusion while enhancing patient safety and not increasing risk of surgical site infection.

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Competing Interests

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End-tidal Anesthetic Concentration: Monitoring, Interpretation, and Clinical Application

Jan F. A. Hendrickx, M.D., Ph.D., Andre M. De Wolf, M.D.

Currently, monitoring during general anesthesia maintained with a volatile anesthetic includes real-time measurement of its end-tidal partial pressure (F_{ET}), displayed on the workstation as concentration and often also as fraction of minimum alveolar concentration (fMAC) or age-adjusted fMAC. The current review explains how proper understanding of the minimum alveolar concentration (MAC) and fMAC concept will help the clinician to titrate volatile anesthetics. Table 1 lists the most frequently used abbreviations and acronyms. Figures and dosing examples are mainly used to explain concepts; we clearly mention whether a figure or part of a figure is based on patient data.

Definition, Determination, Semantics, and Properties of MAC

MAC has been defined by Ted Eger and Lawrence Saidman[1,2] as the steady-state minimal alveolar concentration that results in immobility in 50% of animals and humans after application of a noxious stimulus. In animals, to determine MAC, they are anesthetized with the study drug in the absence of any other drug while keeping the F_{ET} constant for at least 15 min. A strong stimulus (tail clamp or electrical current) is applied for up to 1 min; the head, torso, and limbs are observed for movement, resulting in a quantal response, *i.e.*, movement or no movement. Stiffening, coughing, and hyperventilation are not considered to be movement. If no movement occurs, the F_{ET} is decreased by 20%, but if movement does occur, the F_{ET} is increased by 20%, and the process is repeated once or twice. Smaller up and down changes in F_{ET} allow for more accuracy but will prolong the study. Eventually, the highest F_{ET} that does not prevent movement and the lowest one that does prevent movement are determined, and the F_{ET} "midway" (the term used by Eger) between the brackets is the MAC in that *individual* animal. This process is called the "bracketing technique." Studying a *group* of animals or humans this way allows the determination of the *median* end-tidal concentration that results in immobility in 50% of the subjects of this species, or MAC. At the same time, this technique gives us an estimate of the variability of MAC.

In humans, usually only one observation is made in each patient during initial skin incision after F_{ET} has been held constant for 15 min. If a patient shows no response to the noxious stimulus, the next patient receives a lower F_{ET}; if the patient does respond, then the next patient receives a higher F_{ET} ("up-and-down" method). Each response is plotted as 0 (movement) or 1 (no movement) *versus* F_{ET} (quantal concentration – response curve). Applying logistic regression to this data set yields an S-shaped graph that displays the probability of no response *versus* the equilibrated F_{ET} in the population (fig. 1). The *median* point on the graph represents the F_{ET} that corresponds to the 50% probability of no movement; this specific F_{ET} was called the minimal alveolar concentration (often abbreviated as MAC) by Eger but could also be called the effective concentration that results in a 50% probability of the clinical endpoint being attained in a population, or EC50. This method is called the "quantal technique," although both bracketing and the quantal technique use quantal data. The two analyses result in the same MAC values.[3]

MAC thus is inherently probabilistic: the EC50 of the quantal concentration–response curve generated from a sample of the population cannot be used to predict an all or none response in the individual patient. It is only possible to estimate the *probability* of an individual to display no response at a certain fMAC. It has been determined that the probability of no response at a certain fMAC in the population can be converted into $P_{\text{no-response}}$ in the individual patient: if, at a certain fMAC, 40% of the patients move on incision, then the probability that the individual patient will move is also 40%.[3]

The term "MAC" is plagued with semantic issues. First, there is significant debate on whether the "M" in MAC should refer to "minimal" or "median." When using the bracketing technique in a group of subjects or patients, the "M" in MAC does not represent "minimal"; instead, it is

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Table 1. Definitions, Abbreviations, and Acronyms

Term	Definition
Quantal data	Concentration–response pairs for binary responses (*e.g.*, yes or no; conscious or unconscious)
Effective concentration of quantal data (*e.g.*, EC50, EC95)	EC50 = effective concentration that results in a 50% *probability* of the clinical endpoint being attained in a population
Quantal concentration–response relationship	Describes the relationship between drug concentration and drug effect, which is binary (present or absent), obtained *via* logistic regression
Graded data	Concentration–response pairs for responses that can be quantitated (*e.g.*, percentage of decrease in blood pressure, change in temperature); also called continuous data
Effective concentration of graded data (*e.g.*, EC_{50}, EC_{95})	EC_{50} = concentration at which 50% of maximum *effect* is observed
Graded concentration–response relationship	Relationship between concentration and effect in an individual or in a population (graded data)
Effective dose (*e.g.*, ED50)	ED50 = dose that gives the desired effect in 50% of the study population (only used if plasma concentration is not determined); frequently used incorrectly instead of EC50
F_{ET}	End-tidal gas fraction, expressed as fraction, concentration, or partial pressure (mmHg)
MAC	Median F_{ET} where, after skin incision, 50% of patients do not move (after 15 min equilibration; $MAC_{immobility}$), which reflects potency of the volatile anesthetic; although the acronym originally stood for minimal alveolar concentration, the better definition would be median end-tidal partial pressure; MAC *itself* is not quantal, but it is a descriptive metric determined from quantal outcomes (endpoints) in a population; MAC is equivalent to EC50 for the specific endpoints: MAC_{awake} = $MAC_{unconscious}$ (based on loss of response to verbal command), and MAC_{BAR} (blunting of adrenergic responses, "quantalized" by Roizen *et al.*[20] as a less than 10% rise in systolic blood pressure, heart rate, pupil diameter, or serum norepinephrine after a noxious stimulus)
Potency	F_{ET} of a drug required to produce 50% of maximum drug effect (graded concentration–response relationship) or the F_{ET} at which 50% of study population exhibit the specific quantal effect (quantal concentration–response relationship)
fMAC: fraction of MAC	The end-tidal concentration expressed as a fraction of MAC
Hysteresis	Delay between change in F_{ET} and target tissue partial pressure
Time constant (τ)	The parameter characterizing the time required for an exponentially increasing or decreasing process to change by 63.2% (*e.g.*, change in F_{CNS} of a volatile anesthetic after a step change in F_{ET})
Isobologram	The graphical representation of the concentrations of two drugs, alone and in combination, that produce the same effect or response probability

the *median* of a collection of MAC values, each determined in individual subjects or patients. When using the quantal technique, MAC is calculated by logistic regression as the EC50 value resulting in 50% of the population affected (fig. 1). In either technique, it is the "median" alveolar concentration that is determined, and therefore MAC should be (re)defined as the "median alveolar concentration." If one wants to avoid the issue of minimal *versus* median completely, then MAC could be defined as "the alveolar concentration of a volatile anesthetic at 1 atm at equilibrium that results in a 50% probability of movement in subjects exposed to a standardized noxious stimulus." Second, the term "partial pressure" might be preferred instead of "concentration." For example, the partial pressure at sea level and at high altitude to anesthetize the same patient will be the same, but the concentration will differ because atmospheric pressure will differ. However, because they are related *via* Henry's law and for uniformity, "concentration" will still be used throughout this article. Third, it is not an alveolar concentration but an end-tidal concentration: "ideal alveolar concentration" and end-tidal concentration differ due to dead space ventilation and other factors.[4] This, however, does not invalidate the MAC concept. Fourth, MAC should specify its endpoint ($MAC_{immobility}$). When MAC was initially used as an indicator of anesthetic potency, the only response that was looked for was movement after a noxious stimulus. In addition, the clinical "no response" is not used consistently: MAC_{awake} should be $MAC_{unconsciousness}$.

The Effect of Age on MAC

By definition, the only factor that affects MAC is age (and possibly genetic factors) because MAC is determined in healthy subjects not receiving any other drugs. In humans, MAC increases by 30% from birth until 1 to 6 months of age and then decreases by about 6 to 7% every decade after 20 years. fMAC or age-adjusted fMAC is now displayed on many anesthesia workstation screens (Eger called this "multiples of MAC").[5] After reviewing the algorithms for age correction of MAC by several different anesthesia machine manufacturers, we have concluded that these result in differences as high as 23%.[6] Hereafter in the article, MAC and fMAC are always presumed to be age-adjusted.

Definition and Description of General Anesthesia

Most anesthesiologists currently define general anesthesia as a drug-induced reversible state of unconsciousness while providing appropriate surgical conditions (immobility) with blunting of excessive autonomic responses (BAR) to noxious stimuli.[7,8] Each of these components is mediated by different neural circuits in the central nervous system (CNS). For example, volatile anesthetics mediate

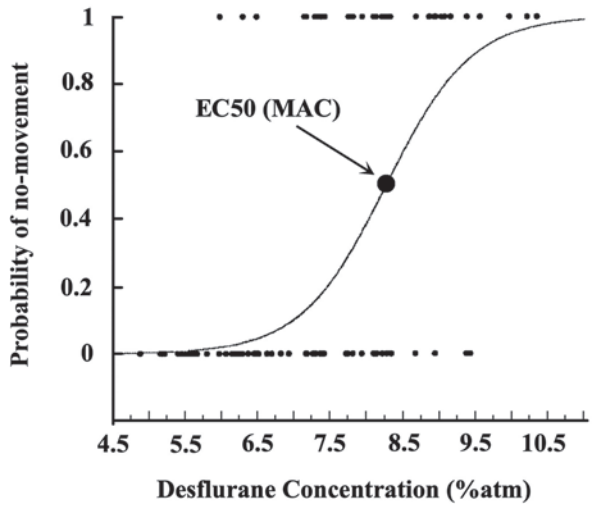

Fig. 1. Quantal concentration–response curve for desflurane. The target response is no movement. During general anesthesia in mice (n = 370), a tail clamp is applied, and movement is observed. Movement in each animal is plotted as 0, and no movement is plotted as 1. The data are fit by logistic regression, resulting in a sigmoid curve. Minimum alveolar concentration (MAC; more correctly called $MAC_{immobility}$) is defined as the median of the concentration–response curve and can also be called the 50% effective concentration (EC50). The figure is based on the work of Sonner.[3] (Sonner JM: Issues in the design and interpretation of minimum alveolar anesthetic concentration (MAC) studies. Anesth Analg 2002; 95:609–14. Adapted with permission.)

immobility mainly at the level of the spinal cord and unconsciousness at the cortical level and the thalamus.[8,9] Analgesia and amnesia are not separate goals of general anesthesia: the unconscious state in and by itself prevents not just the perception of pain (which requires consciousness) but also the formation of memory.[8] It is important to distinguish nociception from pain: nociception is the activation of certain neuronal pathways through the sensory system, while pain is the conscious subjective experience of this "nociceptive information."[8,10] Because all three components of the anesthetic state (unconsciousness, immobility, and blunting of adrenergic responses) can be achieved by volatile anesthetics alone at different but clinically relevant concentrations, these drugs are considered to be complete anesthetics.

While immobility and blunting of adrenergic responses are straightforward to monitor, determining whether the patient is unconscious during general anesthesia is more complex.[11] If unconsciousness is defined as absence of intraoperative awareness, the literature will offer the clinician little dosing guidance because existing, underpowered studies on the incidence of intraoperative awareness mostly rely on *postoperative* interviews or surveys and thus reflect the incidence of intraoperative awareness *with explicit recall*. The low incidence of recall in these studies is not unexpected because memory is more sensitive to general anesthetics than consciousness. Clinicians actually see this every day: patients are clearly conscious during or just after extubation but often have no recall of this event when interviewed later.

Investigators have attempted to determine the presence of "intraoperative awareness" with the isolated forearm test (or technique).[11,12] After induction of anesthesia but before administering neuromuscular blocking drugs, a forearm is isolated with a tourniquet to avoid paralysis of the forearm. The patient is then asked to squeeze the unparalyzed hand. The main advantage of the isolated forearm test is that it does not depend on memory and thus allows us to study the state of consciousness during general anesthesia. In a prospective study of 260 patients, positive responses were reported in 12 patients (4.6%), indicating "perceptive awareness" at that moment.[12] Most positive responders do not move their hand without being instructed ("spontaneously unresponsive"),[13] but very few of those who move have recall for the event.[12,13] It is possible that several of the nonresponders simply lack motivation to obey the verbal command during the isolated forearm test, and therefore "perceptive awareness" may have a higher incidence than these studies suggest. The neuromuscular blocking state increases the probability of a positive response to a verbal command, and volatile anesthetics may reduce it.[12,14,15] Positive responders have a higher incidence of sympathetic activation.[12]

Other observations during these isolated forearm test studies may be very relevant. Bispectral Index (BIS) values are poorly predictive of the isolated forearm test response: even though slightly higher in responders, BIS values of less than 40 occurred in both responders and nonresponders.[12] There is also no difference in the electroencephalogram (EEG) patterns of the frontal lobe between responders and nonresponders.[14] Unfortunately, in these studies, the anesthetic management was not standardized, and very little information about anesthetic technique was provided.

While the clinical relevance of a positive isolated forearm test remains unclear, it corroborates the view held by many cognitive neuroscientists that consciousness *versus* unconsciousness is not a binary phenomenon.[11,13] There may be several degrees of wakefulness and unconsciousness: a patient may be "somewhat" conscious but sufficiently impaired to tolerate surgery yet still be able to respond to a command, all followed by absence of explicit recall.[16] Whether intraoperative awareness without explicit recall harms the patient remains unclear.[12] The isolated forearm test has been applied in a very small number of patients, and in routine clinical care, it is at present impossible to know with certainty whether the fully paralyzed patient is conscious or not. By extension, we cannot know with a high degree of certainty the partial pressure that prevents intraoperative awareness in the individual patient.

This discussion makes it clear that the definition of general anesthesia needs to be adjusted as scientific progress is being made in understanding unconsciousness. With our current understanding, anesthesia can be defined as a drug-induced reversible state of unconsciousness or altered consciousness without explicit recall while providing appropriate surgical conditions (immobility) with blunting of excessive autonomic responses (BAR) to noxious stimuli. In other words, the majority of patients are unconscious, but a minority (4.6 to 6.7%) has reduced consciousness without explicit recall.[12,14] We apparently cannot use frontal EEG parameters or BIS to differentiate between the two groups, and we also do not know whether this reduced conscious state is the result of insufficient anesthesia.

To summarize, the clinician is left with having to titrate drugs to attain unconsciousness or an altered state of consciousness without explicit recall. At present, the marker most often used to help target this state in clinical studies and for which dose–response curves have been constructed is the loss of response to verbal command, discussed in the following section. In what follows, "unconsciousness" is therefore functionally defined as "loss of response to verbal command." However, it is important to realize that any functional definition of "unconsciousness" as mediated by volatile anesthetics will be hampered by our current lack of understanding of unconsciousness itself. Obviously much more research including a much larger number of subjects is needed in this area. This may include the use of more advanced EEG analyses.

Relationship between fMAC and Clinical Effect

The concentration–response relationships between the steady-state fMAC (or F_{ET}) of volatile anesthetics and each clinical effect (unconsciousness, immobility, and blunting of adrenergic responses) can only be constructed after defining each clinical endpoint in terms of a stimulus–response pair indicative of that clinical effect: loss of response to verbal command, loss of movement after incision, and attenuation of heart rate and blood pressure increase after laryngoscopy. The resulting concentration–response relationships are analogous to the concentration–response relationship of intravenous drugs: each curve is described by the effective concentration that results in a 50% probability of the clinical endpoint being attained in a population, called EC50 (fig. 2), and by its slope (Hill coefficient). For volatile anesthetics, the F_{ET} in a population at which there is a 50% chance of no response to a verbal command ($P_{unconsciousness}$ = 50%), no movement after surgical incision ($P_{immobility}$ = 50%), and achieving blunting of adrenergic responses (P_{BAR} = 50%) have been defined as MAC_{awake}, MAC, and MAC_{BAR} by Ted Eger and Larry Saidman, Robert Stoelting, and Mike Roizen, respectively (fig. 2A).[2,17–20] Compared to IV anesthetics, the F_{ET}–response curves have a steep slope with a small variability (SD approximately 0.1).[3,21,22] A SD of 0.1 (or 10%) is less than what is frequently stated (approximately 15%), meaning that the concentration–response curves are actually steeper than often assumed. The other consequence is that approximately 97.5% of patients are immobile with fMAC of 1.2 (steady state). While the reason for this small variability remains a mystery,[23] this distinct important property results in the usefulness of fMAC as an indicator of probability of no response, which is distinctly different from IV anesthetics. The concentration–response curves for unconsciousness, immobility, and blunting of adrenergic responses are often presumed to run parallel, even though this has never been studied in great detail; this is an area that requires more research.

MAC is probabilistic, and thus if we desire to guide clinical use of anesthetics based on fMAC monitoring, we must choose target probabilities for the different clinical endpoints. It is a priority to achieve unconsciousness or altered consciousness without explicit recall with near certainty—it is part of the contract we make with patients when we inform them that they will receive a general anesthetic. If not detrimental (*e.g.*, eye or intracranial surgery), occasional mild movement is acceptable. Finally, some degree of short-lived hypertension and tachycardia is acceptable with some exceptions. To illustrate how the probabilistic nature of MAC can guide the clinician to titrate volatile anesthetics, we propose that general anesthesia in probability space requires a 99.99% or higher, 95%, and 85% likelihood of having attained unconsciousness, immobility, and blunting of adrenergic responses, respectively (fig. 2B). While it could be argued that a probability of unconsciousness of greater than or equal to 99.99% is extremely high and not achievable, Pandit *et al.*[24] have reported an incidence of intraoperative awareness of 0.005%. While this number may underestimate the true incidence because it was obtained through an audit, it does support the order of magnitude of $P \geq 99.99$ as clinically relevant and achievable.[24] Determining target probabilities is what the clinician does intuitively when targeting an fMAC or F_{ET} of the volatile anesthetic. It also allows us to introduce the concept of isoboles that are nothing but lines of equal no-response probability in the presence of certain drug combinations (see "Drug Interactions: Opioids"). The higher the fMAC, the more reassurance the clinician has that a response will be suppressed.

With the current state of knowledge, determining the specific threshold for unconsciousness remains impossible for reasons explained under "Definition and Description of General Anesthesia." In addition, we do not have enough data to construct high-resolution concentration–response curves, especially not at their "tails." Still, thresholds have been recommended that can serve as lower limit alarms in an attempt to at least minimize the occurrence of awareness with explicit recall. Avidan *et al.* in 2011[25] reported that F_{ET} monitoring with the intention to maintain age-adjusted fMAC of 0.7 or higher resulted in a lower incidence of intraoperative awareness than when using an EEG-based

monitor (0.07% *vs.* 0.24%, respectively). The incidence of awareness could even have been lower because fMAC greater than or equal to 0.7 was achieved only a median of 84.8% of the time. Although this study was underpowered, it serves as a guidance for the clinician. While Eger and Sonner recommended 0.5 fMAC to virtually guarantee unconsciousness,[26] they also noticed that learning of emotionally charged information is suppressed at anesthetic concentrations of 1.5 to 2 times MAC_{awake} (0.6 to 0.7 fMAC) and that fMAC greater than 0.6 suppresses explicit and implicit learning.[27,28] Our preference is to err on the higher side (greater than or equal to 0.7 MAC) because it is well tolerated, without hemodynamic compromise in most patients, and without affecting neurologic outcome (*i.e.*, no evidence exists that lowering fMAC from 0.7 to 0.5 would convey a clinically meaningful difference in postoperative outcome). Immobility and blunting of adrenergic response can be achieved at much higher levels of fMAC, but when opioids are coadministered, fMAC needs to be only slightly higher than 0.7 (see "Drug Interactions: Opioids").

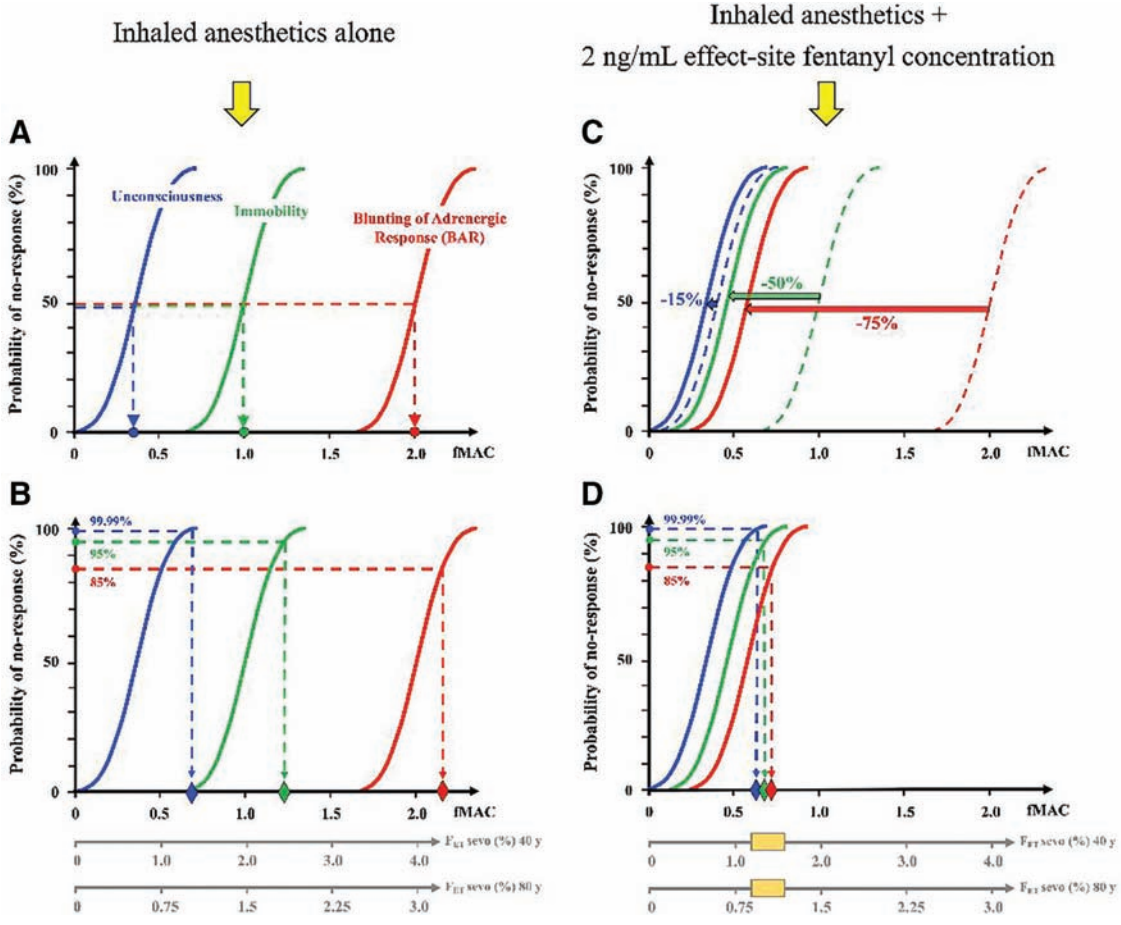

Fig. 2. The quantal concentration–response curves of the steady-state end-tidal partial pressure (F_{ET}) of volatile anesthetics. All curves are meant to explain concepts and are not directly based on patient data, except when specifically mentioned. (*A*) As the concentration (expressed as a fraction of minimum alveolar concentration [fMAC]) is increased, the probability of rendering patients unconscious (*blue*), immobile (*green*), and blunting autonomic responses (BAR) (*red*) increases and reaches 50% at MAC_{awake}, minimum alveolar concentration (MAC), and MAC_{BAR}, respectively (*colored circles* on the *x*-axis). (*B*) We propose that anesthesia requires a 99.99% or greater, 95%, and 85% probability of unconsciousness, immobility, and blunting of adrenergic responses, respectively. The corresponding required steady-state fMAC can be back-extrapolated to the *x*-axis. (*C*) Effect of a 2-ng/ml effect-site fentanyl concentration on fMAC required to ensure the three different clinical endpoints. Opioids do not reduce MAC (a fixed point in the *x*-axis) but reduce the steady-state F_{ET} (or fMAC) needed to attain the same no-response probability ($P_{no\text{-}response}$). This graph is adapted from data by Katoh *et al.*,[30–32] with the percentages being only approximate changes used for illustrative purposes. (*D*) If a 2-ng/ml effect site fentanyl (or equivalent) concentration is maintained, 0.7 fMAC can ensure that all three clinical goals are achieved. Although based on patient data (in the work of Katoh *et al.*[30–32]), this cartoon only illustrates the general concept. (Katoh T, Ikeda K: The effects of fentanyl on sevoflurane requirements for loss of consciousness and skin incision. ANESTHESIOLOGY 1998; 88:18–24; and Katoh T, Kobayashi S, Suzuki A, Iwamoto T, Bito H, Ikeda K: The effect of fentanyl on sevoflurane requirements for somatic and sympathetic responses to surgical incision. ANESTHESIOLOGY 1999; 90:398–405. Adapted with permission.)

However, when immobility or blunting of adrenergic response is critical for an optimal outcome, other drugs (neuromuscular blocking or vasoactive drugs) should be used. fMAC monitoring will help the clinician in avoiding the use of excessive concentrations by applying proper age correction and by correlating the prevailing fMAC with the individual patient response.

In summary, we pragmatically propose that we should aim to achieve probabilities for unconsciousness, immobility, and blunting of adrenergic response of 99.99% or higher, 95%, and 85%, respectively. We also propose that a high probability of unconsciousness may be obtained by administering an fMAC of greater than or equal to 0.7 (age-adjusted, steady-state). Based on the currently available literature, about 5% of our patients during "general anesthesia" may not be completely unconscious but are in a state of altered consciousness without explicit recall, and it is currently impossible to say how this state of altered consciousness should be managed. Consequently, the goals we proposed will likely change when more clinical studies have improved our knowledge.

Drug Interactions: Opioids

In clinical practice, unconsciousness, immobility, and blunting of adrenergic responses are most often obtained through a combination of volatile anesthetics, neuromuscular blocking drugs, and opioids. The use of opioids has profound implications for the predictive value of fMAC because opioids shift each fMAC/$P_{no\text{-}response}$ curve to the left (fig. 2C).[29–31] While it is often claimed that opioids "reduce MAC," they do not—the fMAC to attain a certain $P_{no\text{-}response}$ is reduced, but MAC remains unaltered (it only depends on age).

The degree to which opioids reduce fMAC required to attain a certain $P_{no\text{-}response}$ differs quantitatively for the different clinical endpoints and are described by isobolograms (fig. 3). An isobologram visualizes all possible effect site concentration and/or partial pressure combinations (that are at steady state by definition) of one or more hypnotics or volatile anesthetics with one or more opioids that result in the same $P_{no\text{-}response}$ (fig. 3).[29–32] Of the many possible combinations, we select one in this review to illustrate how opioids differentially affect fMAC for the different clinical endpoints, namely the interaction between sevoflurane and a commonly used fentanyl effect site concentration (C_e) of 2 ng/ml (based on data from Katoh *et al.*).[30–32] At 2 ng/ml C_e fentanyl, the fMAC needed to attain $P_{unconsciousness}$, $P_{immobility}$, and P_{BAR} at 50% is reduced by about 10 to 15%, 50%, and 75%, respectively, causing the three curves to move fairly close together (fig. 2, C and D). An equipotent concentration of another opioid has the same effect: 1.67 ng/ml fentanyl = 0.14 ng/ml sufentanil = 28.8 ng/ml alfentanil = 1.37 ng/ml remifentanil.[33] If for illustrative purposes we continue to define anesthesia in probability space as a 99.99%, 95%, and 85% likelihood of having attained unconsciousness, immobility, and blunting of autonomic responses, isobolographic analysis informs us that combining an fMAC of 0.7 of a volatile anesthetic (yellow circle) with a C_e of 2 ng/ml fentanyl can achieve these goals (fig. 3).[30–32,34] Clinical responses (movement, autonomic responses) in the individual patient will inform the clinician that fMAC or the opioid C_e may have to be increased or decreased, or whether alternatively additional intravenous hypnotics, muscle relaxants, and vasoactive drugs can be used to achieve the desired effects. The probabilistic nature of fMAC implies that it cannot be used with certainty to predict stimulus-response suppression in the individual but can allow the clinician to get an idea of where in the population the individual patient is located and can provide a reference value to guide future dosing.

The differential effect of opioids on the fMAC needed to attain the three different anesthetic endpoints has implications for the use of fMAC as a tool to guide drug dosing. The pronounced effect of opioids on the fMAC–response curves for immobility and blunting of adrenergic responses adds a great deal of uncertainty to appropriate anesthetic dosing for these endpoints. We speculate that this may cause the use of $MAC_{immobility}$ and MAC_{BAR} to be perceived as flawed or useless. In addition, immobility and blunting of adrenergic responses can be accomplished by other drugs like neuromuscular blocking drugs or antihypertensive drugs, making the use of fMAC to attain these goals moot. However, because opioids only minimally shift the fMAC–response curve for unconsciousness to the left, fMAC remains a very valuable indicator of $P_{unconsciousness}$, even in the presence of opioids. The value of fMAC in predicting $P_{unconsciousness}$ is only degraded when other intravenous *hypnotics* are added (*e.g.*, propofol, whose concentration cannot be measured routinely online) while fMAC is starting to decrease to less than 0.7. Whenever fMAC has to be decreased less than 0.7, determining depth of anesthesia by EEG- or EEG-derived indices may be advisable.

Hysteresis

Steady State and Time Constant

For our purposes, hysteresis is defined as the delay between changes in F_{ET} and changes in the partial pressure of anesthetic drug in the target tissue, the CNS. Thus, during initial delivery (wash-in) of volatile anesthetics, F_{ET} is higher than the CNS partial pressure, and during wash-out of anesthetic, F_{ET} is lower than the CNS partial pressure. The physiologic basis for estimating the duration of hysteresis for different volatile anesthetics is described below.

The dose–response curves described assume that F_{ET} has been constant for 15 min ("steady state"). Why is there a requirement for this steady state? The 15-min interval dates back to the halothane era: it is the time it takes for the halothane partial pressure in the CNS (F_{CNS}) to reach 95% equilibration (steady state) with that in the arterial blood (F_a; approximated by F_{ET}).

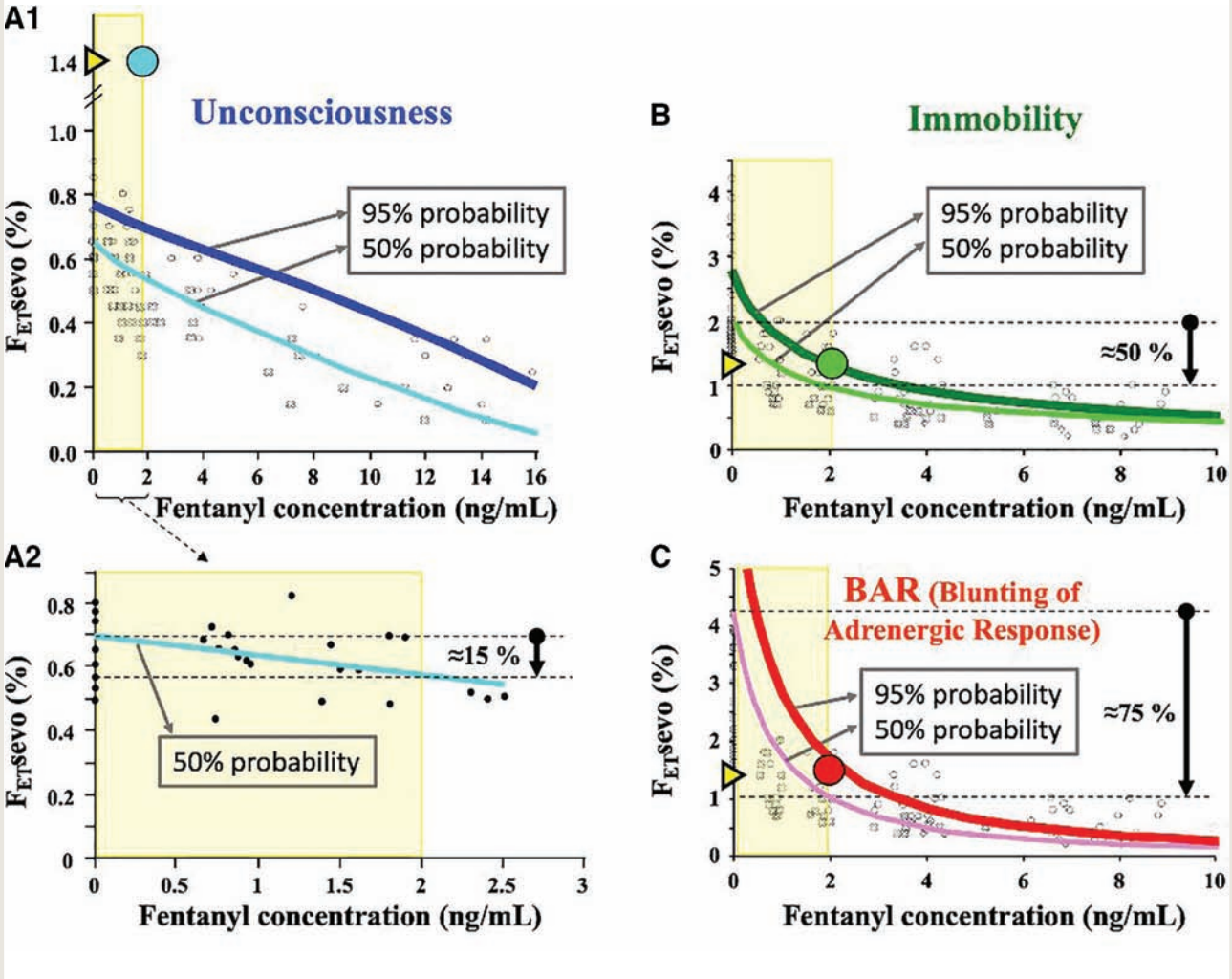

Fig. 3. Differential synergistic effects between opioids and volatile anesthetics. Isoboles describe the same probabilities of response suppression when different concentrations of volatile anesthetics and opioids are simultaneously administered. (*A1* and *A2*) The isoboles representing 50% and 95% probability of unconsciousness are colored *light blue* (*thin line*) and *dark blue* (*thick line*), respectively (95% probability only shown in *A1*). (*B*) The isoboles representing 50% and 95% probability of immobility are colored *light green* (*thin line*) and *dark green* (*thick line*), respectively. (*C*) The isoboles representing 50% and 95% probability of hemodynamic stability are *pink* (*thin line*) and *red* (*thick line*), respectively. For example, at a plasma concentration of ≈2 ng/ml fentanyl (the *yellow-shaded area* represents the 0 to 2 ng/ml range), fentanyl reduces the F_{ET} required to achieve 50% probability of unconsciousness, immobility, and blunting of adrenergic responses by about 10 to 15%, 50%, and 75%, respectively (*thick black arrows*). The combination of 1.4% end-tidal partial pressure (F_{ET}) sevoflurane and 2 ng/ml fentanyl has a 95% probability of providing immobility (*light green circle*), an 85% probability of providing heart rate and blood pressure control (*red circle*), and 99.99% or higher probability of providing unconsciousness (only shown in *A1*). Note the different scaling of the *x*- and *y*-axes in the different panels. This drug combination during steady state thus results in a very high likelihood of having attained $P_{unconsciousness}$, $P_{immobility}$, and P_{BAR} of 99.99% or higher, greater than 95%, and greater than 85%, respectively. Because MAC_{awake} is only minimally influenced by the presence of opioids, we can predict that fraction of minimum alveolar concentration (fMAC) is a very valuable indicator of $P_{unconsciousness}$ at any time. See text for details. The figure was modified after the work of Katoh *et al.*[30–32] (Katoh T, Ikeda K: The effects of fentanyl on sevoflurane requirements for loss of consciousness and skin incision. ANESTHESIOLOGY 1998; 88:18–24; and Katoh T, Kobayashi S, Suzuki A, Iwamoto T, Bito H, Ikeda K: The effect of fentanyl on sevoflurane requirements for somatic and sympathetic responses to surgical incision. ANESTHESIOLOGY 1999; 90:398–405. Adapted and reprinted with permission.)

The time it takes for F_{CNS} to equilibrate with F_a is different for each volatile anesthetic and can be calculated through the concept of the time constant (τ, expressed in units of time). τ describes the exponential rise and fall of the anesthetic partial pressure in the CNS after a step change in F_a. For example, during wash-in (fig. 4A), this is mathematically expressed as

$$F_{CNS} = F_a(1 - e^{-t/\tau})$$

This exponential process is 63%, 86%, and 95% complete after 1, 2, and 3τ. For many exponential processes, τ is calculated as volume divided by flow, but in this case, τ_{CNS}

is calculated as the capacity of the CNS to hold volatile anesthetic divided by its transport toward the CNS with the arterial blood.

$$\tau_{CNS} = (\textbf{capacity}) / (\textbf{volatile anesthetic transport})$$

The capacity (amount of volatile anesthetic the CNS can hold) is determined by the F_a, tissue/gas partition coefficient ($\lambda_{CNS/G}$), and CNS volume (V_{CNS}):

$$\textbf{Capacity} = F_a \times \lambda_{CNS/G} \times V_{CNS}$$

The volatile anesthetic transport to the CNS is determined by the F_a, blood/gas partition coefficient ($\lambda_{B/G}$), and CNS blood flow (Q_{CNS}):

$$\textbf{Volatile anesthetic transport} = F_a \times \lambda_{B/G} \times Q_{CNS}$$

This allows us to calculate τ_{CNS}:

$$\tau_{CNS} = (F_a \times \lambda_{CNS/G} \times V_{CNS}) / (F_a \times \lambda_{B/G} \times Q_{CNS}) \textbf{ or}$$
$$\tau_{CNS} = (\lambda_{CNS/G} \times V_{CNS}) / (\lambda_{B/G} \times Q_{CNS})$$

Because $\lambda_{CNS/G}$/ $\lambda_{B/G}$ can be redefined as $\lambda_{CNS/B}$, the formula can also be written as

$$\tau_{CNS} = (\lambda_{CNS/B} \times V_{CNS}) / Q_{CNS}$$

Using data readily available from the literature, τ_{CNS} for isoflurane, sevoflurane, and desflurane can be calculated to be 3.3, 3.5, and 2.6 min, respectively (exact numbers may vary depending on the data source). Because 95% equilibration requires three time constants, it will take the CNS 9.9, 10.5, and 7.8 min to equilibrate with the isoflurane, sevoflurane, and desflurane F_a, respectively (fig. 4B). Wash-out of volatile anesthetic during emergence is similarly affected by these time constants. It is important to realize that these are only concepts: for example, brain tissue is not a homogeneous tissue, and perfusion is not equally distributed. Nevertheless, the observed emergence times are compatible with the calculated time constants.[35]

fMAC Use during Non–steady-state Conditions: Hysteresis-corrected fMAC

Through routine measurement of F_{ET}, displayed as fMAC value, we can estimate the $P_{no\text{-}response}$ as long as there is a steady-state situation. However, when there is no steady state, fMAC values poorly reflect the concurrent probability of no response. To illustrate, consider two situations in which the same fMAC value is associated with two very different $P_{no\text{-}response}$ values due to hysteresis (fig. 5). During anesthetic wash-in, fMAC poorly reflects the concurrent probability of no response, as it suggests a lower probability of movement in response to surgical stimuli than is clinically observed (in unparalyzed patients). The situation is reversed at the end of an anesthetic when fMAC falls below $MAC_{unconsciousness}$, yet most patients remain unconscious. This may give the uninformed user the (false) impression that the fMAC monitor is clinically useless.

Hysteresis can be taken into account in clinical practice by including the time delay required to reach equilibration (3τ). Converting fMAC to hysteresis-corrected fMAC is mathematically straightforward. Figure 5 displays the course of the fMAC and the hysteresis-corrected fMAC, which is the calculated fMAC in brain tissue. Such a display, pioneered for volatile anesthetics by Kennedy *et al.*,[36] has been commercialized as an individual parameter (MAC Brain, Getinge, Sweden), and the concept has been incorporated into the SmartPilot (Dräger, Germany) and the Navigator (GE Healthcare, USA). Such a display could also guide the clinician who is using an fMAC that is temporarily higher than the target F_{CNS} to speed up the process of increasing F_{CNS} ("overpressure technique"). Similarly, it could guide the tapering process in preparation for emergence.

Hysteresis-corrected fMAC converts fMAC derived from non–steady-state F_{ET} measurements into the real-time $P_{no\text{-}response}$. However, while time for equilibration between F_a and F_{CNS} is an important factor affecting hysteresis, other factors such as neural inertia (a resistance to changes in neural state) play a role as well.[37] In addition, abrupt transitions in brain activity at a constant anesthetic concentration have been observed as well, further confounding hysteresis.[38] It is clear that more research is needed to confirm the findings by Kennedy *et al.*[36] in a much larger and diverse patient population, while further development of the visual displays is ongoing.

Pragmatic Approach to Achieve the Anesthetic State

There are several methods to help the clinician with drug dosing to achieve the anesthetic state. The main goal is to titrate drugs in such a manner that a very high probability of unconsciousness is obtained without overdosing because excessive concentrations can cause hypotension, reduced cardiac output, and excessive CNS depression with possibly increased incidence of postoperative delirium.[39]

One anesthetic dose guiding tool is based on monitoring of drug-induced CNS depression, which results in unconsciousness. This can be done through raw EEG observation, EEG analysis such as BIS determination, or auditory evoked potential analysis. The advantage of these monitoring techniques is that they can be applied during inhaled anesthesia, as well as intravenous anesthesia, and with or without opioids or other intravenous anesthetics or adjuvants. Disadvantages include the proprietary algorithms, the processing delay, and the lack of exact thresholds that guarantee unconsciousness.

Although MAC *itself* is not quantal, it is a descriptive metric determined from quantal outcomes (endpoints) in a population. The quantal and probabilistic properties of fMAC, combined with consideration of hysteresis and drug interactions, guide the titration of volatile anesthetics

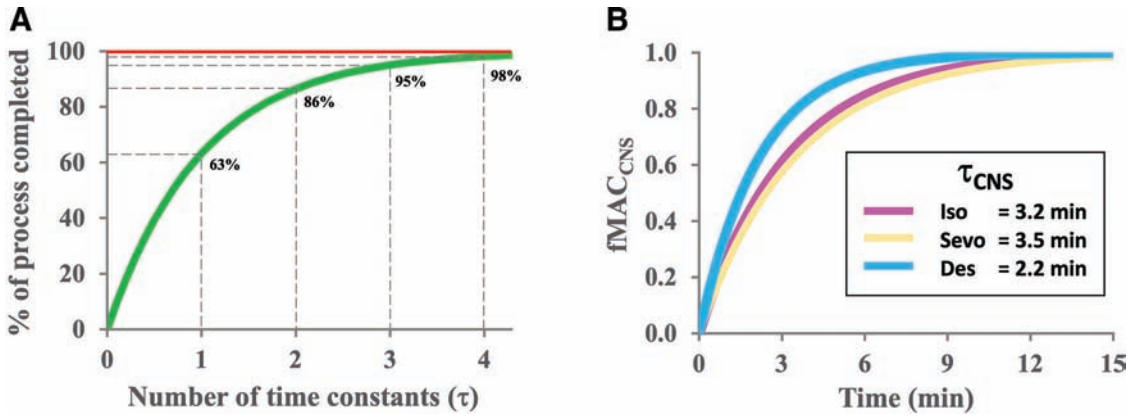

Fig. 4. Mathematical description of organ wash-in. (*A*) The time required to wash in a compartment is determined by the time constant (τ). This exponential process is 63%, 86%, and 95% complete after 1, 2, and 3τ. See text for details. (*B*) Central nervous system (CNS) time constant (τ_{CNS}) defines the exponential rise and fall of the partial pressure in the CNS after a step change in F_a. For example, during wash-in, F_{CNS} at time $t = F_a (1 - e^{-t/\tau})$. Color coding: τ_{CNS} for isoflurane (Iso, *pink*), sevoflurane (Sevo, *yellow*), and desflurane (Des, *blue*). See text for details.

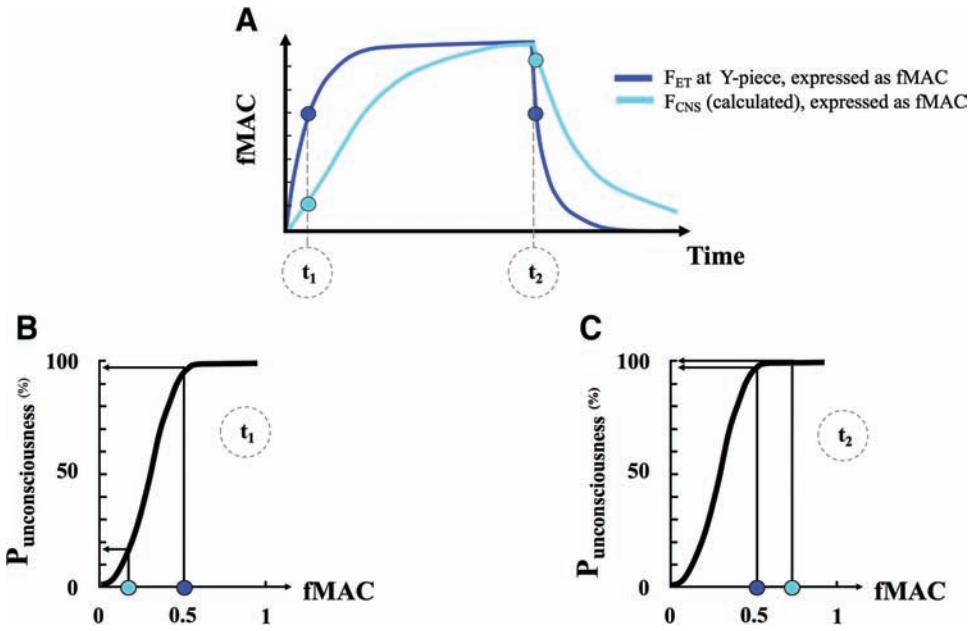

Fig. 5. Hysteresis-corrected fraction of minimum alveolar concentration (fMAC): use of fMAC during non–steady-state conditions. Most contemporary monitors display an age-adjusted fMAC value calculated directly from the measured end-tidal partial pressure (F_{ET}) (*dark blue line*). However, this fMAC value is also displayed when there is no steady state (*A*; at the beginning and at the end of the anesthetic, F_{ET} poorly reflects F_{CNS}). The partial pressure in the CNS (F_{CNS}, *light blue line*, expressed as fMAC) trails F_{ET} during wash-in (t_1) and wash-out (t_2), a phenomenon called hysteresis. This causes identical fMAC values (*dark blue circles*) to represent two different probabilities of no-responsiveness: *B* shows that F_{CNS} is lower than suggested by F_{ET} at the beginning of the anesthetic, and *C* illustrates that F_{CNS} is higher than suggested by F_{ET} after the anesthetic is discontinued. This may cause the clinician to get the impression that the fMAC concept does not work. See text for details.

to achieve the proper anesthetic state. With any volatile anesthetic, we propose that keeping fMAC greater than or equal to 0.7 helps to minimize the risk of consciousness or awareness with explicit recall while at the same time reducing the incidence of severe overdosing. Understanding the effect of hysteresis and drug interaction with opioids

and intravenous hypnotics can help to avoid inadequate anesthesia immediately after anesthesia induction when the propofol concentration is decreasing and the partial pressure of the volatile anesthetics in the CNS is gradually rising, which otherwise could result in a "valley of potentially inadequate anesthesia." Modern technology will assist us during this transition (see "The Future of MAC: Drug Advisory Displays").

Increasing fMAC to greater than or equal to 1 to prevent or treat movement or excessive adrenergic responses to nociceptive stimulation is rarely necessary because these goals can be accomplished by additional opioid administration or the use of other drugs (neuromuscular blocking drugs, β-antagonists, vasodilators, among others). Intraoperative opioid administration is still the mainstay of our anesthetic management[40] and is very effective in achieving a high probability of no movement (even without neuromuscular blocking drugs) and blunting of adrenergic responses.

MAC is useful because it describes the potency of the different volatile anesthetics in a unifying manner. Because MAC reflects the probability of movement after a noxious stimulus, MAC is forever linked to probability of immobility.[41] However, this is not the main reason fMAC is useful: the main benefit from the MAC concept is that the available evidence suggests that maintaining fMAC greater than or equal to 0.7 is currently our single best line of defense against intraoperative consciousness with explicit recall when using volatile anesthetics, a topic that warrants further study.[3] In clinical practice, at steady state (or with hysteresis correction) and in the absence of other drugs, fMAC also provides us with a reference value against which the response of the individual patient can be benchmarked against that of the population and be used as the patient's own reference to guide future drug dosing. In the presence of opioids, we now understand that the fMAC to attain these probabilities needs to be adjusted. Displaying fMAC on the anesthesia machine thus has significant advantages over displaying only F_{ET}.

The Future of MAC: Drug Advisory Displays

Should we abandon the use of the acronym "MAC" and use a new denotation that is conceptually and semantically more correct and aligns our clinical pharmacology terminology with that of intravenous drugs? Would doing so add any real clinical value? In our opinion, at this moment, the term "MAC" should not be not replaced by EC50, mainly because the displayed fMAC applies to all volatile anesthetics, thereby reassuring the clinician with one look at the monitor that the risk of postoperative explicit recall becomes very small if the volatile anesthetic is titrated to a hysteresis-adjusted fMAC of 0.7 or higher.[42,43] fMAC also allows the same lower alarm limit to be applied to all volatile anesthetics. Showing the hysteresis-corrected fMAC provides the same information when there is no steady state. As a result, the presentation of the fMAC value on the anesthesia machine, a drug advisory display avant la lettre, will continue to serve us for a while to come.

In the presence of opioids, fMAC monitoring continues to be helpful to ensure unconsciousness but is much less valuable to determine the probability of immobility and blunting of adrenergic responses. In addition, if intravenous hypnotics (*e.g.*, propofol) are added to the anesthetic mix, then even estimating the probability of unconsciousness becomes problematic. This is where advanced drug advisory displays (SmartPilot and Navigator) may become useful. The first generation of these devices calculates probabilities and their time course when a mixture of volatile anesthetic, propofol, and the most readily available opioids are used. The first clinical studies with the SmartPilot are encouraging and ongoing.[44,45] Future versions will very likely also handle other intravenous drugs such as ketamine, dexmedetomidine, and lidocaine. Until these smarter drug advisory displays are available, EEG-based monitors may be advisable when an intravenous hypnotic drug is used. The use of a combination of drug dosing advisory displays and EEG-based monitors in particular deserves further study.

Conclusions

Volatile anesthetics, combined with opioids, continue to be the most widely used drugs to maintain general anesthesia. The most important value of displaying fMAC lies in minimizing the incidence of awareness with explicit recall by ensuring unconsciousness or altered consciousness without explicit recall with a very high probability. While there is no absolute lower cutoff value that guarantees unconsciousness or altered consciousness without explicit recall, based on the limited available evidence, we propose a steady-state fMAC greater than or equal to 0.7, and in the absence of steady state, a computer-calculated hysteresis-corrected fMAC can be used. fMAC is less useful to titrate volatile anesthetics to obtain immobility and blunting of the autonomic nervous system response because of pronounced drug interactions with opioids (and other drugs). Advanced drug advisory displays require more development and research on how they could be helpful to titrate volatile anesthetics, opioids, and other drugs to ensure unconsciousness and immobility without neuromuscular blocking drugs. Whether more sophisticated EEG-based depth-of-anesthesia monitors will completely replace or rather supplement hysteresis-corrected fMAC and advanced drug advisory displays is unclear at this moment.

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Competing Interests

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ANESTHESIOLOGY

Historical and Modern Evidence for the Role of Reward Circuitry in Emergence

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ABSTRACT

Increasing evidence supports a role for brain reward circuitry in modulating arousal along with emergence from anesthesia. Emergence remains an important frontier for investigation, since no drug exists in clinical practice to initiate rapid and smooth emergence. This review discusses clinical and preclinical evidence indicating a role for two brain regions classically considered integral components of the mesolimbic brain reward circuitry, the ventral tegmental area and the nucleus accumbens, in emergence from propofol and volatile anesthesia. Then there is a description of modern systems neuroscience approaches to neural circuit investigations that will help span the large gap between preclinical and clinical investigation with the shared aim of developing therapies to promote rapid emergence without agitation or delirium. This article proposes that neuroscientists include models of whole-brain network activity in future studies to inform the translational value of preclinical investigations and foster productive dialogues with clinician anesthesiologists.

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Emergence from general anesthesia is a dynamic time of transition from the anesthetized state to the awake state that continues to be an unpredictable and fragile period for patients in perioperative care.[1–3] Some evidence suggests that emergence is regulated by additional neural processes independent of drug clearance[4–6] and is not simply the reverse process of anesthetic induction, as described by models of anesthetic hysteresis.[7–9] Many surgical cases necessitate rapid arousal for assessment of patients' postoperative cognitive and motor abilities. Therefore, rapid and smooth emergence is desirable for both patient safety and perioperative efficiency. Why some patients emerge quickly from higher anesthetic concentrations than others and why subsets of patients undergo an agitated, combative state during the process of emergence is currently not well understood at multiple levels. Together, this unpredictability and the lack of an established therapy to facilitate emergence highlight the need for a better understanding of the basic neuropharmacologic mechanisms mediating emergence from anesthesia.

Emergence agitation can be dangerous, with patients manifesting combative behaviors that can result in self-injury, harm to providers, catheter removal, self-extubation, and airway obstruction. Postoperative delirium can also result in longer hospital length of stay and worsened clinical outcomes.[10–12] Efforts to mitigate adverse emergence phenomena, like agitation and delirium, are currently focused on avoiding inhalational anesthetics or supplementing inhalational agents with intravenous sedatives like the α2 receptor agonist dexmedetomidine.[13–18]

However, the results from these studies vary widely with patient population,[19,20] and the basic neuronal mechanisms modulating emergence under different anesthetic conditions are still unclear. Mechanisms of emergence from volatile or propofol anesthesia, which can directly bind to γ-aminobutyric acid (GABA) receptors, can be inconsistent with mechanisms found to mediate the effects of ketamine, which blocks glutamatergic neurotransmission.[21,22] However, common anesthetic substrates in the brain exist, such as the activation of hypothalamic neurons.[23] Here, we will focus our discussion on studies that use propofol, sevoflurane, or isoflurane for maintenance of general anesthesia, since they form much of the literature investigating mesolimbic circuitry in emergence. Further mechanistic basic science research is needed to examine whether findings hold true across disparate anesthetic conditions. There is a clinical need for therapeutic interventions targeting emergence and the postanesthetic period to improve the predictability, speed, and safety of anesthesia care. By prioritizing a translational and multidisciplinary approach, basic neuroscientists can help to uncover these gaps in knowledge.

Decades of accumulating literature support a role for dopaminergic signaling through the brain reward circuitry in promoting arousal (see reviews[24–26]). Here, we summarize key clinical and preclinical evidence supporting a central role for the brain's mesolimbic dopaminergic reward circuitry in modulating emergence from general propofol and volatile anesthesia, with a focus on the ventral tegmental area and

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the nucleus accumbens regions. The same neural circuitry may be important for the pathophysiology of emergence agitation, given the essential role of reward circuitry in regulating emotional and arousal-related behavioral states. We then discuss systems neuroscience approaches for bridging preclinical and clinical studies of brain reward circuitry in emergence to promote the therapeutic application of preclinical investigations (fig. 1).

Until recently, high-resolution approaches for careful examination within the intact brain did not exist to enable discrete cell type– and region-specific investigations of circuit dynamics. However, now we can harness viral-mediated and genetic approaches to deliver engineered photoactivatable compounds and perform whole-brain imaging at single-cell resolution. This more intricate systems neuroscience approach can be used to understand brain circuitry in preclinical models of awake, behaving rodents and even nonhuman primates.[27–30] To place the granularity of these investigations in a clinically useful framework, mechanistic cellular-level studies must then be examined in the context of changes to whole-brain activity.

While there is a broad and vast literature describing the role of dopaminergic circuitry in mediating arousal and reward-reinforcing behaviors, this review is limited to systems neuroscience studies of particular relevance to the clinical practicing anesthesiologist (table 1). We also refer

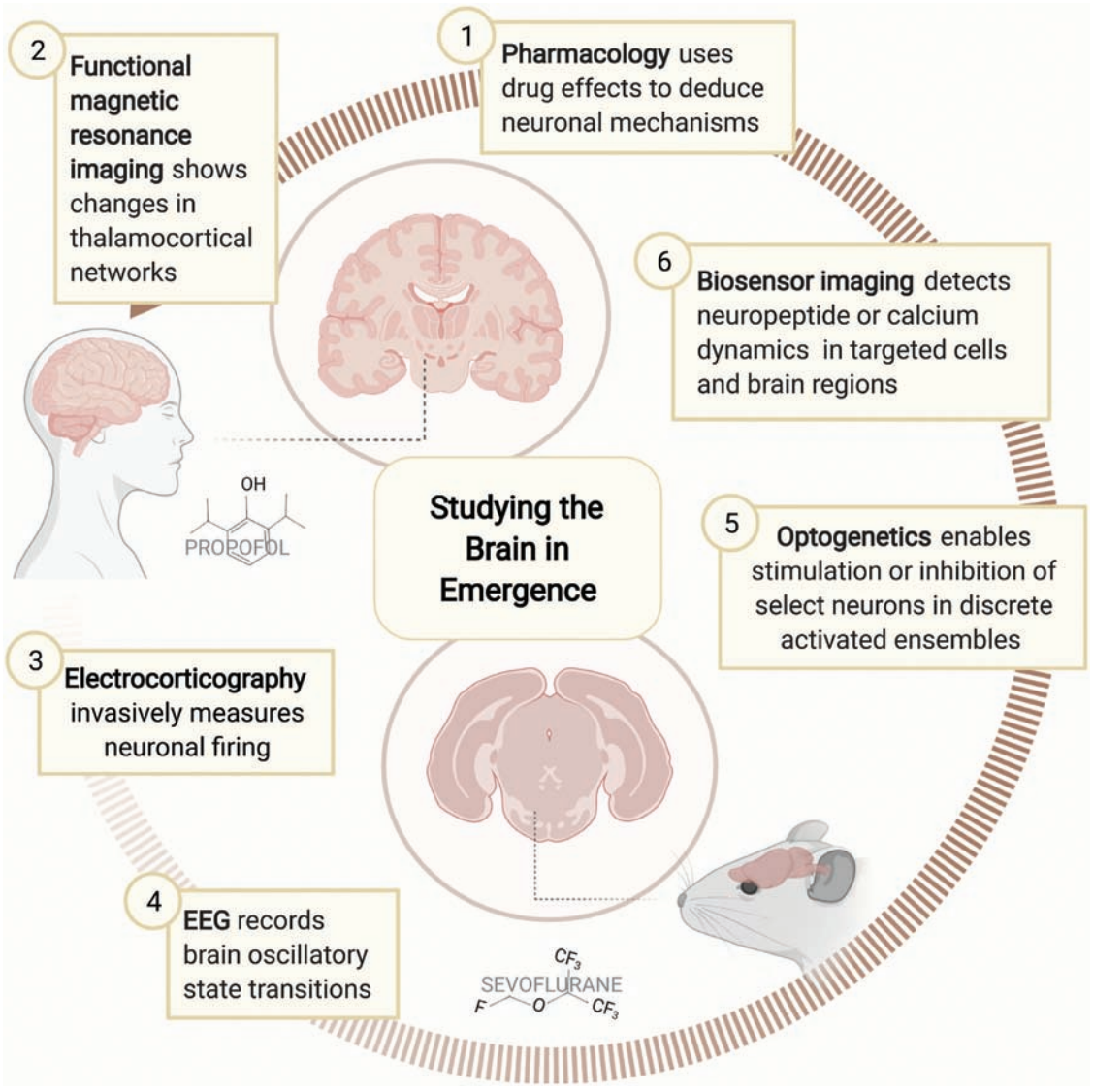

Fig. 1. Schematic highlighting the need to bridge the gap between preclinical and clinical studies of anesthesia emergence with translational research. The *numbered boxes* highlight common methodologies in either clinical or preclinical research. The *insets* show midbrain slices from the human and mouse brain, as well as the chemical structures of propofol and sevoflurane, two of the most common anesthetics under investigation. The figure was created with BioRender.com. EEG, electroencephalogram.

Table 1. Selected List of Historical References, Primary Preclinical Articles Investigating the Role of Mesolimbic Circuitry in Emergence, and Suggested Literature Reviews on Emergence

Citation	Brain Region/Neurotransmitter	Anesthetic	Species	Major Finding
Historical references				
Eckenhoff *et al.* (1961)[1]		Thiopental, halothane, ether, cyclopropane, nitrous oxide, spinal	Human	First descriptions of "emergence excitement" and differential effects of anesthetic drug treatment in 14,436 patients
Mantz *et al.* (1994)[31]	Striatum/dopamine	Halothane, isoflurane, thiopental, ketamine	Rat	Anesthetics significantly alter spontaneous and evoked dopamine release in striatal synaptosomes
Irifune *et al.* (1997)[32]	Nucleus accumbens/dopamine	Isoflurane	Mouse	HPLC assays show increased dopamine turnover, hyperlocomotion during emergence
Tsukada *et al.* (1999)[33]	Striatum/dopamine	Isoflurane	Rhesus monkey	Positron emission tomography and microdialysis show enhanced DAT inhibition and D2 receptor binding under isoflurane
Fiset *et al.* (1999)[34]	Medial thalamus, midbrain	Propofol	Human	Positron emission tomography shows cerebral blood flow changes in midbrain and thalamus
Brain region–specific manipulations				
Kelz *et al.* (2008)[35]	Hypothalamus/orexin	Isoflurane, sevoflurane	Mouse	Ablation of orexinergic neurons or an orexin-1 antagonist delays emergence, not induction
Mhuircheartaigh *et al.* (2010)[36]	Putamen, thalamus, cortex	Propofol	Human	Functional magnetic resonance imaging blood-oxygen-level–dependent imaging shows changes in subcortical connectivity
Shirasaka *et al.* (2011)[37]	Prefrontal cortex/orexin	Propofol	Rat	ICV injection of orexin speeds emergence, increases norepinephrine and dopamine release in the prefrontal cortex
Solt *et al.* (2014)[38]	Ventral tegmental area, substantia nigra/dopamine?	Isoflurane, propofol	Rat	Electrical stimulation of the ventral tegmental area speeds emergence
McCarren *et al.* (2014)[39]	Ventrolateral preoptic nucleus/norepinephrine	Isoflurane, dexmedetomidine	Mouse	Single-cell reverse transcription–polymerase chain reaction of VLPO neurons and role of adrenergic manipulation
Vazey and Aston-Jones (2014)[40]	Locus coeruleus/norepinephrine	Isoflurane	Rat	Chemogenetic activation of locus coeruleus neurons speeds emergence
Zhou *et al.* (2015)[41]	Ventral tegmental area/dopamine	Propofol, isoflurane, ketamine	Rat	Lesioning ventral tegmental area dopamine neurons with 6-hydroxydopamine prolongs emergence from propofol, not isoflurane
Taylor *et al.* (2016)[42]	Ventral tegmental area/dopamine	Isoflurane	Mouse	Optogenetic activation of ventral tegmental area dopamine neurons speeds emergence
Muindi *et al.* (2016)[21]	Parabrachial nucleus/glutamate?	Isoflurane	Mouse	Electrical stimulation of parabrachial nucleus speeds emergence
Fu *et al.* (2017)[43]	Central medial thalamus/norepinephrine	Propofol	Rat	Norepinephrine microinjection in central medial thalamus speeds emergence
Du *et al.* (2018)[44]	Locus coeruleus/norepinephrine	Propofol, etomidate	Zebrafish	Deletion of dopamine-β-hydroxylase in locus coeruleus neurons delays emergence from intravenous anesthesia
Yin *et al.* (2019)[45]	Ventral tegmental area, hypothalamus/GABA	Isoflurane	Mouse	Activation of ventral tegmental area GABA to hypothalamus slows emergence
Wang *et al.* (2019)[46]	Parabrachial nucleus/glutamate	Sevoflurane	Mouse	Activation of parabrachial nucleus glutamate neurons speeds emergence
Zhang *et al.* (2019)[47]	Reticular thalamus/norepinephrine	Propofol	Mouse	Locus coeruleus-to-TRN norepinephrine projections delay emergency by activating α1 adrenergic receptor
Torturo *et al.* (2019)[48]	Ventral tegmental area/dopamine	Isoflurane	Rat	Isoflurane inhibits exocytosis in cultured rat dopamine neurons by a distinct calcium-mediated mechanism
Li *et al.* (2019)[49]	Ventral tegmental area/orexin	Isoflurane	Rat	Microinjection of orexin in the ventral tegmental area promotes emergence by activating dopamine neurons
Zhang *et al.* (2020)[50]	Prefrontal cortex/acetylcholine, adenosine, norepinephrine	Isoflurane	Mouse	Microdialysis studies showing neurotransmitter roles in anesthetized-to-awake state transition
Luo *et al.* (2020)[51]	Basal forebrain/acetylcholine	Isoflurane, propofol	Mouse	Chemogenetic activation of cholinergic neurons speeds emergence
Gretenkord *et al.* (2020)[52]	Ventral tegmental area, prefrontal cortex/dopamine	Urethane	Rat	Stimulation of ventral tegmental area and D1 receptors in prefrontal cortex promotes arousal
Ao *et al.* (2021)[53]	Paraventricular thalamus/dopamine	Isoflurane	Mouse	PVT cFos activity increases after emergence, enhanced by a D2 agonist
Zhang *et al.* (2021)[54]	Nucleus accumbens shell/dopamine	Isoflurane	Mouse	D1 receptor agonist accelerates emergence in young but not aged mice
Bao *et al.* (2021)[55]	Nucleus accumbens/ dopamine	Sevoflurane	Mouse	Chemogenetic activation of D1 receptors delays induction and accelerates emergence
Recommended literature reviews on emergence				
Franks (2008)[56]			Human/rodent	Molecular targets of arousal
Brown *et al.* (2010)[57]			Human	Relationship of anesthesia to sleep and coma
Tarnal *et al.* (2016)[5]			Human	Hysteresis, neural inertia, and active emergence
Kelz *et al.* (2019)[4]			Rodent	Neurotransmitter modulators of emergence

DAT, dopamine transporter; GABA, γ-aminobutyric acid; HPLC, high-pressure liquid chromatography; ICV, intracerebroventricular; PVT, paraventricular thalamus; TRN, thalamic reticular nucleus; VLPO, ventrolateral preoptic nucleus.

the reader to comprehensive reviews of mesolimbic circuitry[58–62] and to articles discussing transcriptomic tools for studying the brain under anesthesia,[63,64] such as single-cell RNA sequencing and clustered regularly interspaced short palindromic repeats/Cas9 approaches[65–67] that are beyond the scope of this review.

Brain Reward Circuitry

The brain reward circuitry, also known as the mesolimbic dopamine system or mesolimbic circuitry, is composed of interconnected subcortical and cortical brain regions. This circuitry is evolutionarily conserved across mammals to mediate reinforcing behaviors important for survival, like sex[68,69] and food consumption.[70–80] The same brain regions also modulate sleep/wake transitions and states of arousal essential for executing these reward-related behaviors.[81–85] Dysregulation of reward seeking is concomitant with dysregulated arousal.[86] For example, disordered sleep is an important feature of illnesses characterized by anhedonia and dysregulated reward circuit functioning like depression, addiction, schizophrenia, and Parkinson's disease.[87,88]

Dopamine neurons in the ventral tegmental area project to the nucleus accumbens, forming a key projection in the mesolimbic dopamine system, the circuitry that guides reward-related behaviors and promotes arousal (fig. 2).[58,89–98] The nucleus accumbens is a central processing hub in ventral striatum that integrates inputs from the ventral tegmental area with inputs from myriad brain regions in the reward circuitry, which has been shown to guide a variety of behavioral responses and emotional states.[58,62,70,91,99–103] Distinct neurochemical markers in the striatum divide its neurons into either the direct ("go") or indirect ("no-go") pathways.[58] These markers include the dopamine receptors, which are G-protein–coupled receptors classified as either D1 (G_s-coupled) receptors that signal through the direct pathway or D2 (G_i-coupled) receptors that signal through the indirect pathway. The direct pathway, named for its direct projection to the ventral tegmental area in the midbrain, expresses the neuropeptides dynorphin and substance P, while indirect pathway peptide expression includes enkephalin and adenosine A2A receptors.[58] This region is very heterogenous in anatomical and functional properties and is enriched in numerous neuropeptides, and modulators include acetylcholine, among others.[58,70,97,99,100,104] The dorsal striatum expresses similar dopamine receptor subtypes as ventral striatum but projects to the substantia nigra, instead of the ventral tegmental area, to guide motor responses to stimuli. In reality, the canonical role of dorsal striatum as confined to pure sensorimotor processing and the role of ventral striatum as confined to emotional processing are less explicitly segregated than was once believed (see review[105]).

γ-Aminobutyric acid–mediated (GABAergic) medium spiny neurons are the principal neurons in the nucleus accumbens, comprising over 95% of the neuronal population, and form local inhibitory synapses between medium spiny neurons, as well as long-range GABA projections to other brain regions in mesolimbic reward circuitry (fig. 2). Approximately 3% of nucleus accumbens neurons are cholinergic interneurons releasing acetylcholine, while less than 2% are inhibitory interneurons releasing GABA with either somatostatin or parvalbumin.[58,106] As a result, the inhibitory GABA receptors are ubiquitously expressed in nucleus accumbens in addition to neuronal expression of dopamine receptors, μ-opioid receptors, glutamate receptors, enkephalin, dynorphin, and other neuropeptide signaling substrates. The mechanism of action of propofol and volatile anesthetics, like sevoflurane and isoflurane, is known to involve direct activation of GABA type A receptors (see review[107]). Binding of propofol or volatile anesthetic to GABA receptors presumably may occur at both the medium spiny neuron and interneuron, thus modulating dopaminergic signaling within the nucleus accumbens microcircuit in addition to affecting long-range GABAergic projections in mesolimbic circuitry. Dopamine signaling also changes calcium currents and *N*-methyl-D-aspartate–induced currents studied in striatal slices.[108–111] Changes in dopamine release, dopamine D1 receptor activation, and transcriptional activation of deltaFosB in nucleus accumbens are shown to be necessary for the behavioral and abuse liability properties of propofol administration.[112–115] Further research is needed to define GABA, glutamate, and dopaminergic interactions during behavioral emergence.

Ventral tegmental area dopamine neuron firing patterns signal errors in reward prediction, help to guide and modify behavior, and reinforce the motivation to seek rewards.[70–74,116–119] Ventral tegmental area dopamine neurons respond in two different modes: single-spike tonic firing to maintain dopamine tone[120–122] and phasic burst firing that is thought to signal an unexpected reward or salient event.[72,123–129] Dopamine neuron bursting activity increases during transitions from sleep to wakefulness.[81,82] Increased burst firing from ventral tegmental area neurons results in dopamine release to downstream interconnected brain regions, including the nucleus accumbens, prefrontal cortex, hypothalamus, and amygdala[94,98,130,131] (fig. 2).

Classically attributed to orchestrating dopamine signaling, ventral tegmental area circuitry is modulated by numerous other neuropeptides, as well as local and long-range GABAergic, glutamatergic, serotonergic, and cholinergic projections.[98,132,133] For example, ventral tegmental area GABA neurons send long-range projections to synapses directly on nucleus accumbens cholinergic neurons, forming one specialized circuit important for reward reinforcement[100] and associative learning.[134] Ventral tegmental area GABA neurons are also engaged in sleep arousal and modulated by anesthesia.[135–137] The role of ventral tegmental area GABA to nucleus accumbens cholinergic neuron projections in emergence is unclear. The ventral tegmental area connects directly with the thalamus, basal forebrain, orexinergic neurons in the hypothalamus, noradrenergic neurons

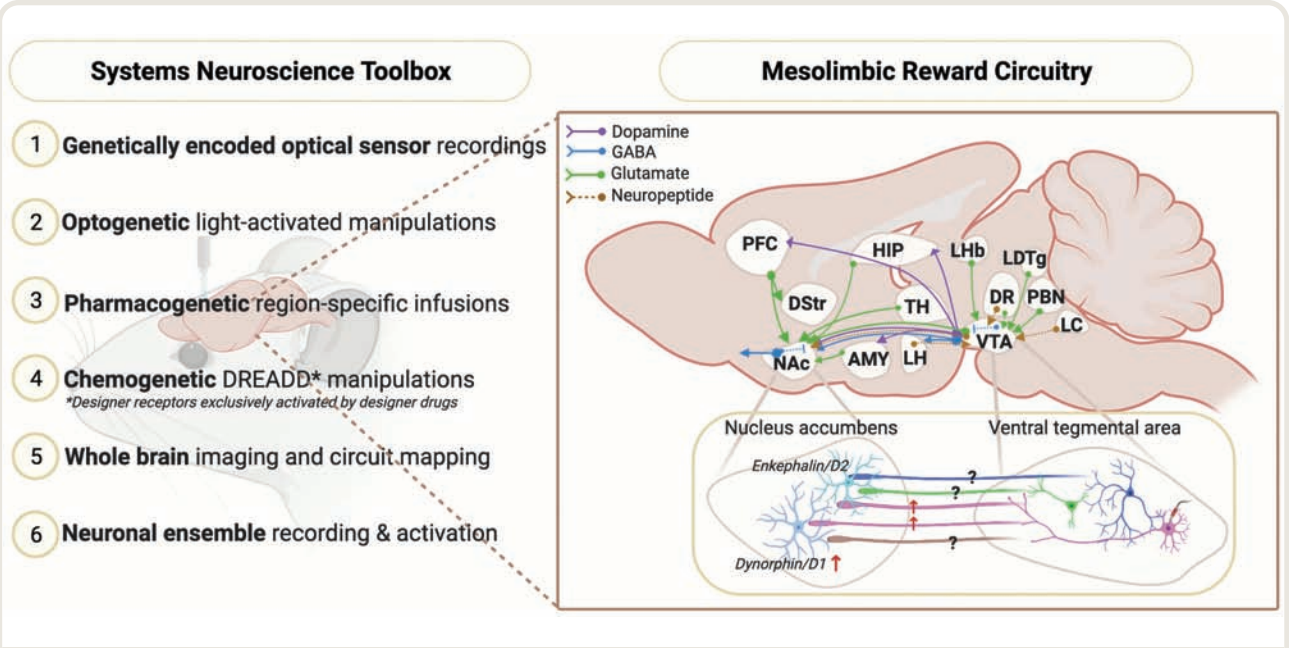

Fig. 2. (*Left*) Systems neuroscience toolbox for dissecting mesolimbic circuitry with *numbered boxes* highlighting key methodologies for circuit-level and whole-brain investigations. (*Right*) Highly simplified schematic of mesolimbic reward circuitry with relevant glutamate (*green arrow*), dopamine (*purple arrow*), γ-aminobutyric acid (GABA; *blue arrow*), and other neuropeptide (*dashed arrow*) projections shown. Serotonergic projections from the dorsal raphe (DR), noradrenergic projections from locus coeruleus (LC), and orexinergic projections from the lateral hypothalamus (LH) (not shown) are also important for emergence. The *inset* below shows a magnified view of the ventral tegmental area (VTA) to nucleus accumbens (NAc) projections with *upward arrows* indicating the increased activity of dopaminergic projections and D1 receptor activation in emergence. The role of the other VTA to NAc projections in emergence is unclear. The figure was created with BioRender.com. AMY, amygdala; DStr, dorsal striatum; HIP, hippocampus; LDTg, lateral dorsal tegmentum; LHb, lateral habenula; PBN, parabrachial nucleus; PFC, prefrontal cortex; TH, thalamus.

in the locus coeruleus, and serotonergic neurons in the dorsal raphe, each of which is individually important for mediating arousal and differentially affected by anesthesia.[4,138,139]

Importantly, the state of general anesthesia, a drug-induced reversible coma, is distinct from natural sleep (see comprehensive reviews on this topic[57,140]). While insight into brain reward circuitry is gained from studies of sleep arousal, the same mechanisms should not be expected to correlate directly with emergence from anesthesia. This important caveat must be considered when comparing studies of sleep arousal and anesthesia as reviewed in this article.

Human Studies of Reward Circuitry in Emergence

Pharmacologic, neuroimaging, and genetic manipulations support the role of monoaminergic circuits in both arousal from sleep and emergence from general anesthesia. Dopamine and norepinephrine are important neurotransmitter mediators of arousal, as evidenced by impaired arousal seen in mice missing the dopamine β-hydroxylase[26,141,142] and dopamine transporter[143] genes. In humans, single-nucleotide gene polymorphisms affecting the dopamine transporter and dopamine D2 receptor genes are associated with variations in self-reported sleep duration.[144] Treatment with a tyrosine hydroxylase inhibitor, with the end result of decreasing dopaminergic tone, increases sleepiness in studies of healthy adults.[145,146] Dopamine D2 receptor levels also decrease specifically in the human ventral striatum after sleep deprivation, as assayed by positron emission tomography imaging.[147]

In contrast, stimulants that increase dopaminergic and catecholaminergic tone have strong effects on arousal. Dopamine-enhancing medications heighten arousal and accelerate emergence, as shown by studies of D1 receptor agonist treatment.[148,149] In contrast, dopamine antagonism with droperidol slows emergence by deepening sevoflurane anesthesia.[150] Together, these studies indicate a role for dopaminergic tone in promoting arousal, with a specific role for activation of dopamine receptors in ventral striatum.

Human neuroimaging under anesthesia consistently demonstrates thalamic deactivation and disruption of thalamocortical connectivity in states of general anesthesia,[151–154] along with deactivation of the basal forebrain and basal ganglia,[36] important components of the brain reward circuitry. Studies using functional magnetic resonance imaging infer changes in brain activity by correlating changes in cerebral

blood flow. However, they are not able to directly measure neuronal activity, an important limitation when interpreting results of functional magnetic resonance imaging studies. Invasive electrocorticography can be used to obtain direct recordings from the cortex of patients undergoing neurosurgery for intractable epilepsy. These studies demonstrate thalamocortical suppression with induction of general anesthesia, while recovery from anesthesia reflects a progressive increase in cortical activity, a decrease in reticulothalamic activity, and a return of tonic activity in the thalamus.[155] General anesthesia also inhibits auditory processing in higher-order auditory association areas while maintaining local field potential neuronal activity in the primary auditory cortex, suggesting that anesthesia may selectively affect higher-order signaling to disrupt cortical circuits.[156]

Anesthesia research in both rodent models and the human clinical population utilize electroencephalogram (EEG) changes and perioperative EEG monitoring as a tool for monitoring the depth of anesthesia. General anesthetics are well known to produce distinct EEG patterns, with a shared pattern of increased delta oscillations (see a recent review[140]). Interestingly, a recent study links increased delta oscillations to dopamine depletion and loss of D2 receptor activation in mouse striatum independent of anesthesia exposure.[159] Studies of D1 dopamine receptor activation during emergence from isoflurane anesthesia in mice show reduced delta and increased gamma oscillations, accelerating emergence.[54,148] EEG changes do not always reliably correlate with behavioral arousal. For example, optogenetic stimulation of ventral tegmental area dopamine neurons promoted behavioral arousal with minimal change in EEG.[42]

EEG studies of the brain are useful as a noninvasive and easily translatable method but have limitations for interpretation. A recent multicenter study enrolled 60 healthy volunteers to evaluate frontal–parietal EEG dynamics in recovery from anesthesia, independent of surgery.[160] Results from this study support a model of early return of prefrontal cortical dynamics and executive function. However, EEG dynamics do not predict cognitive recovery after anesthesia. Burst suppression in the EEG is considered to reflect a very deep state of anesthesia that may be desirable to avoid.[158] In a study of 27 healthy human volunteers, EEG burst suppression does not change the time to emergence or affect the degree of cognitive impairment after isoflurane exposure, using a computational model to predict time of emergence.[161] The Electroencephalography Guidance of Anesthesia to Alleviate Geriatric Syndromes (ENGAGES) randomized clinical trial also finds that EEG-guided administration of general anesthesia does not reduce the incidence of postoperative delirium, compared to usual care, in adults aged 60 yr and older.[162] Thus, while EEG is a useful readout of brain oscillatory arousal states, it is only one tool for evaluating clinical effects of emergence in the perioperative setting. A multidisciplinary systems neuroscience approach, additionally informed by preclinical research, is needed for a holistic view of anesthesia emergence and postanesthetic cognitive sequelae.

Preclinical Studies of Reward Circuitry in Emergence

Current basic neuroscience understanding of arousal is derived primarily from rodent studies of sleep/wake states and general anesthesia.[35,42,46,49,52,85,135,137,138,163] Sleep studies in rodents consistently support a central role for dopaminergic signaling and specifically ventral tegmental area neuron activity in arousal.[26,85,135,137] Emergence from anesthesia, defined as arousal and return of awareness, is assayed behaviorally in the rodent by restoration of the righting reflex response, a reflex that develops shortly after birth to maintain the prone position. In these studies, the mouse or rat is turned on its back while anesthetized, and upon emergence, the animal will right itself to having its paws on the ground.[56,164–167]

Preclinical research supports an important role for dopaminergic signaling in mediating emergence from anesthesia. Isoflurane anesthesia inhibits synaptic vesicle exocytosis from dopamine neurons in cultured rat ventral tegmental area dopamine neurons.[48,168] Subanesthetic propofol exposure causes an increase in spontaneous ventral tegmental area dopamine neuron firing recorded from rat brain slices, and propofol potentiates evoked postsynaptic excitatory synaptic currents recorded downstream in the nucleus accumbens.[169] The stimulant drug amphetamine also causes presynaptic release of dopamine and inhibits dopamine reuptake in the striatum in recordings from striatal brain slices.[170] Systemic administration of methylphenidate and amphetamine, which increase catecholaminergic tone by inhibiting norepinephrine and dopamine reuptake, speeds emergence from both isoflurane and propofol general anesthesia in rodents, as measured behaviorally by restoration of the righting reflex.[171–174] Similarly, other reports show that intravenous caffeine administration also accelerates emergence from isoflurane general anesthesia in both mice and humans.[175–178] These indirect pharmacologic studies support a general role for increased dopaminergic tone influencing emergence.

Studies directly manipulating the ventral tegmental area under general anesthesia demonstrate the sufficiency of ventral tegmental area neuron activity in promoting emergence. Direct stimulation of ventral tegmental area neurons using an electrode inserted above the ventral tegmental area in the rat results in faster emergence from both isoflurane and propofol anesthesia.[38] Further, cell type–specific stimulation of only dopamine neurons in the ventral tegmental area using optogenetics in transgenic mice promotes emergence from isoflurane anesthesia.[42] Together, these findings support a working model of reduced ventral tegmental area dopamine neuron activity under general anesthesia, with emergence characterized by a resurgence of dopamine activity as brought about by direct neuronal stimulation or stimulant drug administration.

Multiple reports indicate a critical role for the engagement and activation of dopamine receptors during emergence. Early studies of phenobarbital anesthesia demonstrate a role for activation of both dopamine D1 and dopamine D2 receptors in promoting emergence using systemic receptor agonist treatment in rats[179,180] and rabbits.[181] Dopamine D1 receptor agonists promote emergence from isoflurane and propofol anesthesia.[148] Administration of A2A receptor agonist, which also activates medium spiny neurons expressing D2 receptors, modulates the depth of propofol anesthesia and activates the nucleus accumbens in mice as measured by increased cFos expression.[182] However, these studies all use systemic administration of dopamine receptor agonists, so the neural circuit mechanism and sites of their action in the brain are unknown. Direct microinjection of D1 receptor agonist or antagonist into the nucleus accumbens supports a bidirectional regulation of time to emergence with dopamine receptor activation specifically within the nucleus accumbens.[54] In addition, selective chemogenetic activation of nucleus accumbens D1 receptor–expressing neurons accelerates emergence and delays induction with sevoflurane.[55]

Together, these findings support a working model of the anesthetized state as marked by a reduction of dopaminergic tone, with emergence from anesthesia promoted by increased ventral tegmental area dopamine neuron activity, which subsequently causes activation of downstream D1-type dopamine receptors within the nucleus accumbens (fig. 2). It is unclear whether the increase in ventral tegmental area activity is driven by increases in tonic dopamine neuron firing or phasic discharge during emergence. While ventral tegmental area dopamine appears to be necessary for emergence, it is unclear whether ventral tegmental area stimulation alone is sufficient to drive emergence. Optogenetic studies of ventral tegmental area dopamine neurons in emergence used repeated stimulation for more than 30 min to increase the probability of righting.[42] Additional mechanisms may be engaged within ventral tegmental area circuitry with repeated stimulation over time. Ventral tegmental area dopamine neurons project to numerous target brain regions to form the mesolimbic reward circuit, as discussed previously. Outside of the ventral tegmental area, manipulations of the parabrachial nucleus, which directly projects to the ventral tegmental area, the locus coeruleus, and the thalamus, also promote emergence from general anesthesia.[40,183–186] It is possible that additional dopaminergic pathways are further engaged in these studies. In addition to dopamine, the ventral tegmental area contains numerous neuropeptide-containing neurons,[70] as well as GABAergic and glutamatergic cells that can send long-range projections. The effects of increased ventral tegmental area neuron activity during emergence on heterogeneous downstream circuitry remains to be fully described (fig. 2, *inset*). Many investigations of brain circuitry also largely ignore the contribution of nonneuronal cell types, while there is a new study indicating an important role for astrocytes in emergence.[187] Future research must be aimed at comprehensively evaluating all cell types in target brain circuit regions during emergence to form a complete mechanistic understanding and provide new therapeutic targets.

Preclinical Neuroscience Methods for Neural Circuit Investigation

Modern neuroscience tools enable a detailed dissection of neural circuits in the awake-behaving animal with high temporal and spatial resolution using optical manipulation and behavioral modeling. Neural circuit investigation is strengthened by an interrogation at multiple levels of analysis, from molecular/cellular to systems to behavioral. Beginning with the revolutionary introduction of optogenetics,[188–190] the optical tools available for interrogating brain circuit connectivity now extend from light-activated ion channels to optically active G-protein–coupled receptors, like parapinopsin[191] and the optogenetically activated μ-opioid receptor[69,146] and β_2 adrenergic receptor.[147] Genetically encoded fluorescent sensors of neuropeptide and neurotransmitter release, such as the dLight sensor, which detects dopamine release, and the GPCR activation-based norepinephrine sensor sensor, which detects norepinephrine release,[195] among many others,[196] are used together with calcium imaging to better elucidate the dynamics of neural circuit action during behavior. Optofluidic devices also enable the wireless light-evoked delivery of drugs into the brain for pharmacologic studies with high temporal and regional specificity.[197–200] Coupled to transcriptomic manipulations at the single-cell level, the investigation of novel receptor-mediated signaling mechanisms in specific brain circuits is possible with exquisite detail.[201]

Advances in microscopy now allow for imaging across the whole brain at single-cell resolution after brain clearing using light sheet microscopy.[202] Some studies of general anesthesia are beginning to take advantage of the whole-brain approach to investigating neural circuits, like the reticular activating system.[203] In addition, calcium dynamics can be imaged at the individual neuronal level within a specified circuit using *in vivo* two-photon microscopy in a head-fixed animal or *in vivo* one-photon imaging after implanting a miniature microscope (graded index lens) in freely moving mice.[204–207] Calcium imaging can then be paired with optogenetic studies to dissect the effects of circuit activation or inhibition on neuronal activity. These newer imaging modalities provide high single-cell and spatial resolution, enabling detailed cellular-level preclinical investigations, compared to approaches with poorer spatial resolution like functional magnetic resonance imaging and positron emission tomography.[208,209]

Multiregion, high-density recordings of neuronal activity using advanced physiology methods like implanted silicone probes, called Neuropixels (imec, Leuven, Belgium), can

be used to decipher circuit activity during emergence.[195] Neuropixels do not utilize genetically encoded sensors and thus lack cell-type specificity, as well as tracking of the same neurons across long-term temporal domains.[210] However, Neuropixels can be paired with optotagging, in which a neuron is optogenetically activated to determine its identity, and the high-density nature of Neuropixels recordings affords a more system-wide view of a given series of brain regions.

Neuronal recordings and optogenetic manipulation can then be paired with computational neuroethology for closed-loop stimulation or analytical studies.[211] Closed-loop deep brain stimulation therapy improved depression symptoms in one individual with major depression,[212] and similar approaches could be adopted for modifying emergence. Open source toolkits for high-throughput behavioral analysis using machine learning approaches, like DeepLabCut,[213] SimBA (https://github.com/sgoldenlab/simba, Golden Lab, University of Washington, Seattle, Washington),[214] or DeepSqueak,[215] can be applied to studying emergence from anesthesia. Machine learning approaches are useful for identifying previously unknown behavioral repertoires within simple behaviors, such as grooming[216] and subtle pharmacologic effects on behavior in rodents.[217] Behavioral models of emergence such as spontaneous restoration of the righting reflex are currently analyzed as a binary output interpreted by visual manual scoring (either positive when the rodent is aroused and upright or negative when the rodent is lying unconscious on its back). The binary restoration of the righting reflex model as currently analyzed is suggested to variably correlate with cortical signatures of arousal assayed by EEG and local field potential analysis.[218] Even a simple experimental model like restoration of the righting reflex presents an array of behavioral features (*e.g.*, whisker movement, tail curling, side rolling, increased chest movement, then righting). In-depth behavioral classification using pose estimation and machine learning classification methods helps to remove experimenter subjectivity and provides an automated analysis pipeline to facilitate data comparisons across experiments, investigators, and research centers. There are several studies applying machine learning approaches to assessing depth of anesthesia in human subjects.[219,220] These approaches may yield further mechanistic insights when also translated to the preclinical model for concurrent neural circuit interrogations.

Conclusions: Bridging the Preclinical–Clinical Divide

To develop a better understanding of anesthetic emergence and work toward new clinical strategies to promote smooth emergence, existing studies of network state changes in humans might be used as working templates for further mechanistic dissection of brain arousal circuitry in preclinical animal models. By layering relevant clinically translational endpoints onto preclinical models, such as EEG analysis and functional magnetic resonance imaging imaging to identify shared areas of activation, a comprehensive view of brain circuitry during emergence may develop. The preclinical model can then be used to develop a more granular mechanistic analysis of neuronal changes, taking advantage of high-resolution single-cell approaches in the context of whole-brain dynamics.

Emergence is likely a convergence of the activity of multiple distributed transmitters, receptors, and circuits, for example, a unification of orexinergic, dopaminergic, and noradrenergic systems.[4] Further investigations are needed to understand the effects of different anesthetic conditions, like ketamine as compared to sevoflurane or propofol, on neural circuits. To study emergence, the aggregate brain network must then be examined using network-wide manipulations. The patterns of neuronal circuit activity that regulate emergence can be directly controlled and modified by utilizing closed-loop approaches, as discussed in this article. Additional tools to elucidate behaviorally activated brain-wide circuits include utilizing transgenic mice, like Fos-CreER^{T2} [221], together with viral approaches to access activity-regulated neuronal ensembles[207,222,223] (see review[224]). At this level of whole-brain analysis and neuronal activity, it is then possible to generate neuronal decoders for predicting and modifying emergence. Overall, an improved understanding of brain circuitry changes during emergence will help to facilitate predictable transitions from the anesthetized state to the awake state that will in turn improve patient safety and satisfaction with anesthesia care.

While it is not easy to reconcile preclinical and clinical approaches, innovative new tools exist for studying brain circuitry that can be applied strategically to heighten the translational value of preclinical anesthesia investigations. We must build dialogue and collaborative studies between basic neuroscientists and clinician researchers, appreciate the limitations of each scientific approach, and compare parallel findings from the preclinical and clinical literature as guides for future shared investigation.

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Competing Interests

The authors declare no competing interests.

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ANESTHESIOLOGY

Advances in Neuroimaging and Monitoring to Defend Cerebral Perfusion in Noncardiac Surgery

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ABSTRACT

Noncardiac surgery conveys a substantial risk of secondary organ dysfunction and injury. Neurocognitive dysfunction and covert stroke are emerging as major forms of perioperative organ dysfunction, but a better understanding of perioperative neurobiology is required to identify effective treatment strategies. The likelihood and severity of perioperative brain injury may be increased by intraoperative hemodynamic dysfunction, tissue hypoperfusion, and a failure to recognize complications early in their development. Advances in neuroimaging and monitoring techniques, including optical, sonographic, and magnetic resonance, have progressed beyond structural imaging and now enable noninvasive assessment of cerebral perfusion, vascular reserve, metabolism, and neurologic function at the bedside. Translation of these imaging methods into the perioperative setting has highlighted several potential avenues to optimize tissue perfusion and deliver neuroprotection. This review introduces the methods, metrics, and evidence underlying emerging optical and magnetic resonance neuroimaging methods and discusses their potential experimental and clinical utility in the setting of noncardiac surgery.

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Perioperative complications associated with noncardiac surgery are a major source of morbidity and mortality, with an estimated 2.5 million deaths from more than 12 million complications globally each year.[1] The brain is particularly vulnerable to perioperative injury. Perioperative brain dysfunction is associated with long-term disability and death and is increasingly recognized as a major complication after surgery.[2] Due to limited capacity for anaerobic metabolism, the brain is susceptible to perioperative injury from hypoperfusion and mismatch between oxygen supply and demand. Anesthesia and surgery are associated with numerous homeostatic disturbances that can affect end-organ perfusion. Drug effects, hypotension, blood volume changes, metabolic and hemostatic dysfunction, inflammation, and the level of oxygen saturation can all cause an imbalance between substrate supply and demand within end organs.[3] Thus, there is a strong physiologic rationale to defend cerebral perfusion, and a range of techniques are now available in the perioperative setting to measure cerebral perfusion, oxygenation, metabolism, and their complications.

It is then curious that despite these strong physiologic underpinnings, no consistent associations between systemic hemodynamics, brain perfusion changes, and perioperative brain injury have been identified after noncardiac surgery.[4] Perioperative hypotension has been implicated in end-organ hypoperfusion and injury in other organ systems, with well-established biomarkers revealing concerning levels of perioperative cardiac (8%) and renal (6%) injury after major surgery.[5,6] The scenario with brain injury is more complex; there are both paucity of available biomarkers for injury and incomplete understanding of the mechanism of injury.

Our understanding of the pathophysiology of perioperative neurologic injury, including both cerebrovascular and neurocognitive disorders, is in its infancy. To date, there are no well-established, clinically relevant biomarkers for

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perioperative brain dysfunction, which limits the ability of clinicians to diagnose the condition, treat patients, and conduct clinical trials.[7] Perioperative neurocognitive disorders can be subtle and nonspecific, clinically silent, or clinically overt syndromes. They can be classified in the following ways: (1) postoperative delirium, an acute, fluctuating disorder of consciousness characterized by inattention and changes in cognition and perception[8]; (2) postoperative cognitive dysfunction, an objective decline in cognition as measured by neuropsychologic tests, which can be early (between postoperative days 1 and 30) or late (after postoperative day 30); or (3) where objective evidence of cognitive decline is combined with clinical cognitive concern, delayed neurocognitive recovery (between postoperative days 1 and 30) or postoperative neurocognitive disorder (between 30 days and 1 yr after surgery).[8] Perioperative stroke is defined as brain infarction of ischemic or hemorrhagic etiology that occurs during surgery or within 30 days after surgery. Covert stroke is a key subcategory that predominately includes smaller ischemic lesions detected by magnetic resonance imaging without significant clinical manifestation.[9]

Although the precise neurobiology of perioperative neurologic injury remains poorly understood, research from both cardiac and noncardiac surgery has shown a range of inflammatory, hemodynamic, and genetic factors are involved.[10,11] Neuroimaging has been essential for improving our understanding of perioperative neurocognitive disorders. Disturbances in cerebral metabolism, perfusion, oxygenation, and activity of functional neural networks can all be detected by neuroimaging and monitoring. Furthermore, various neuroimaging and neuromonitoring techniques, including magnetic resonance imaging, near-infrared spectroscopy, and transcranial Doppler ultrasound, can quantify neurologic insult and potential injury mechanisms in the perioperative clinical environment.

Advances in neuroimaging now offer novel avenues for monitoring the brain as the primary site of injury and as an indicator of adequate organ perfusion. This review describes how evolving noninvasive imaging and neuromonitoring using magnetic resonance, sonographic, and optical techniques can expand our understanding of perioperative organ injury. We discuss how emerging imaging biomarkers for neurologic injury could be used to inform future prevention strategies and potentially reduce the burden of perioperative complications through improved assessment of patient risk, optimized hemodynamic management, and enhanced detection of postoperative neurologic injury. Temporal and spatial constraints of brain measurement techniques are a key consideration. Because of the topographic organization of brain function, small regional differences can dramatically alter the impact of injury. The high metabolic demands of the brain mean that momentary hypoperfusion may result in injury. Hence the ideal neuromonitor would report continuous bedside brain imaging. In this review, we report techniques that may approach this ideal—either *via* serial neuroimaging (magnetic resonance imaging) or continuous bedside neuromonitors that have the ability to deliver spatially resolved measurements/images (transcranial Doppler ultrasound, near-infrared spectroscopy).

Perioperative Complications and Brain Dysfunction

The 2019 Detection and Neurological Impact of Cerebrovascular Events in Noncardiac Surgery Patients: A Cohort Evaluation study (NeuroVISION) reported an alarmingly high incidence of brain injury after noncardiac surgery, with 7% of patients older than 65 yr experiencing postoperative covert stroke.[7] Perioperative neurocognitive disorders are even more frequent, with early postoperative cognitive dysfunction and postoperative delirium reported in 10 to 15% and 20 to 30% of patients after noncardiac surgery, respectively.[12–14] These figures are likely to be conservative as there are methodologic limitations that confound the detection and assessment of these disorders.

Perioperative neurologic complications have a major effect on patient outcomes. Covert stroke is associated with both early postoperative cognitive dysfunction and a 13% increase in cognitive decline at 1 yr.[7,15,16] Overt perioperative stroke conveys a 13.3% risk of 30-day mortality[17] and presents a substantial healthcare burden in the form of lifelong neurologic sequelae in 69% of cases.[18] Postoperative cognitive dysfunction and delirium both delay patient recovery. Data show these conditions double the average length of hospital stay and increase both short- and long-term patient mortality.[19,20] The burden of these complications is worsened by their often covert nature. Only 40 to 50% of patients with delirium are diagnosed and treated, and the majority of ischemic brain lesions fail to present with classic stroke symptoms, yet markedly impair long-term cognitive recovery.[5,19,20]

Anesthesia and surgery may disrupt cerebral perfusion and lead to imbalance between cerebral oxygen supply and demand. Systemic factors such as hypotension or autonomic dysfunction can precipitate hypoperfusion. These hemodynamic disturbances have been a popular proposed treatment target, as cerebral perfusion can be measured, and systemic physiology may be optimized to maintain perfusion—for example, optimizing blood pressure, oxygenation, ventilation (carbon dioxide), and depth of anesthesia. Despite these known physiologic insults, and considerable clinical and research focus, to date, no study has demonstrated conclusive benefit from individualized perioperative hemodynamic optimization.[4] One possible reason for the lack of conclusive data is that these studies are dependent on development of sensitive objective outcome measures for neurologic dysfunction. Another is that, as in the case of brain injury, other mechanisms such as inflammation may dominate.

Advances in neuroimaging and monitoring hold the key to identifying covert, previously undetectable perioperative

organ injury and examining the role of optimizing cerebral perfusion in the perioperative setting. Neuroimaging and monitoring can assess the brain both as the primary site of injury and as an indicator of adequate organ perfusion. The brain is the most metabolically active organ, and its vascular resistance adapts to match blood flow with metabolic requirements in response to both systemic perfusion pressure changes (myogenic cerebral autoregulation) and local neuronal activity (metabolic cerebral autoregulation or neurovascular coupling). These characteristics potentially make the brain a viable index organ for monitoring the effects of systemic physiology on organ microcirculation, cellular metabolism, and oxygenation, in addition to primary brain pathophysiology.[21]

Perioperative Neuroimaging and Neuromonitoring

There is currently no ideal universal neuroimaging or neuromonitoring modality available to reliably characterize cerebral well-being in the perioperative setting. Monitoring is essential to decipher perioperative changes in cerebral hemodynamics, oxygenation metabolism, and function, as these may often be clinically silent. Furthermore, specific tools are needed to identify the functional or structural phenomena that correlate with subtle or unexplained clinical manifestations, as well as clinical syndromes such as delirium, which represent a spectrum of pathophysiology. Historically, such imaging has included positron emission tomography and single photon emission computed tomography; however, as these techniques use ionizing radiation or radioactive tracers, their use is inherently restricted to limit radiation exposure. Continuous assessment with nonionizing, noninvasive techniques such as optical techniques and ultrasonography provide alternative options for cerebral hemodynamic monitoring in the perioperative clinical environment, as does the application of serial neuroimaging methods like magnetic resonance imaging. Advances in the past 5 yr have enabled unique assessment of both neurologic and cerebrovascular function, ranging from direct measurement of cellular metabolism to global functional and brain network imaging *via* connectomics.[22]

All the available modalities have trade-offs between spatial and temporal resolution, and constraints to deliver at the bedside, particularly in the operating room. For example, magnetic resonance imaging creates a snapshot with high spatial resolution but can miss transient or important temporal phenomena, which occur frequently in the perioperative period. In addition, monitoring in the operating room is not generally feasible outside of a dedicated image-guided operating theater or cardiac suite due to safety and practical constraints. Conversely, optical techniques, such as near-infrared spectroscopy, can be performed at the bedside in the various patient environments, including operating rooms and hospital wards. However, spatial resolution is limited, and large, cumbersome monitoring arrays can be needed for data collection.

In the following sections, we outline indices of neurologic and cerebrovascular function that are relevant to perioperative management. This review will focus on emerging techniques that have the capacity to perform multisite measurement/imaging of biomarkers of neurologic injury used to optimize cerebral perfusion: optical, ultrasonographic, and magnetic resonance techniques. We have not included electroencephalography as this has been reviewed extensively elsewhere and does not directly reflect a representation of regional perfusion.[23] A complete summary of the imaging modalities, along with their respective strengths, weaknesses, and limitations, can be found in table 1.

Optical Techniques

Optical techniques have the capability to measure a range of properties of superficial cortical tissue continuously and completely noninvasively at the bedside. *Via* these techniques, it is possible to measure surrogates of cerebral blood flow, as well as cerebral oxygenation, and even cellular oxygenation and/or metabolism. Due to their noninvasive nature and relative portability, optical modalities are particularly useful for continuous monitoring in the intraoperative and/or postoperative environment.

Optical brain monitoring exploits the physical properties of biologic tissues. For example, human tissue is relatively transparent in the near-infrared light spectrum (from 700 to 1,000 nm), which permits the interrogation of superficial cerebral structures. Introduced light diffuses through superficial cerebral structures and is subject to scattering and absorption interactions. Photon-tissue absorption and scattering interactions can be used to identify specific tissue chromophores (hemoglobin and cytochrome oxidase) and physical properties (flow) in the superficial cerebral cortex.

Near-infrared Spectroscopy

Near-infrared spectroscopy is a noninvasive continuous optical technique that can measure the concentration of different tissue chromophores at the bedside. In clinical practice, near-infrared spectroscopy is used to predict the balance between oxygen supply and demand *via* regional hemoglobin–oxygen saturation. As the concentration of specific tissue chromophores is directly proportional to light attenuation, the concentration of chromophores, such as oxyhemoglobin (HbO_2) and deoxyhemoglobin (HHb), is derived using modifications of the Beer–Lambert law. Absolute regional cerebral hemoglobin oxygen saturation ($[HbO_2]/[HbO_2 + HHb]$) is the main parameter reported by clinical devices and is employed as a surrogate reflecting the balance between tissue oxygen supply and demand. Regional cerebral hemoglobin oxygen saturation is derived from the slope of attenuation between two spaced detectors, exploiting simplifications to the diffusion approximation of light transport in tissues.

Table 1. Summary of Tools for Perioperative Assessment of Cerebrovascular Function and Brain Connectivity

Imaging Modality	Category	Mechanism	Applications	Advantages	Disadvantages
Optical techniques					
Near-infrared spectroscopy[24]	Continuous neuromonitoring	Measures oxy- and deoxyhemoglobin to estimate tissue oxygen saturation; concentration of chromophores can be evaluated in superficial tissues by attenuation of light introduced to the head and other body regions; broadband spectroscopy employing a multitude of wavelengths can resolve other chromophores such as cytochrome c oxidase, the terminal electron acceptor in the mitochondrial respiratory chain	Perioperative monitoring of cerebral oxygen saturation	Commercial monitors available; portable or bedside use for continuous monitoring; minimal operator training	Incomplete clinical evidence to direct interventions; interpretation may not be straightforward due to complexity of regional cerebral hemoglobin oxygen saturation number; although inexpensive, cost remains a barrier to use
Diffuse correlation spectroscopy[25]	Continuous neuromonitoring	Measures blood flow in tissue from interference pattern from a time-resolved laser light introduced into the forehead; produces scaled index of cerebral blood flow; can be calibrated with optical indicator dye and Fick principle	Continuous perioperative cerebral blood flow monitoring	Continuous bedside cerebral blood flow monitoring; high accuracy in published studies	Primarily research technique; expensive hardware based on fiberoptics, time-resolved laser, and detector
Diffuse optical tomography[26, 27]	Continuous neuroimaging	Implementation of near-infrared spectroscopy that uses an extended array of optodes to measure attenuation over a large area; optical data can be reconstructed to report topographic or tomographic images of oxy- and deoxyhemoglobin across large regions of the brain	Assessment of functional brain activity, connectivity between brain regions	Only functional neuromonitoring that can be used at the bedside or in theater	Many devices are expensive; currently a research technique; monitoring array can require considerable time to apply and optimize; interpretation requires experience and appropriate data analysis techniques
Ultrasound					
Transcranial Doppler ultrasound[28,29]	Continuous neuromonitoring and neuroimaging	Estimates blood flow through the large intracranial blood vessels based on ultrasound Doppler shift; reports cerebral blood flow velocity, which approximates cerebral blood flow assuming vessel radius is static; two main methods exist: (1) duplex ultrasound, which combines Doppler and B-mode to visualize parenchyma, vascular structures, and magnitude and direction of flow simultaneously, or (2) power-motion Doppler, which combines M-mode and Doppler to offer improved resolution of flow velocity without structural context; three-dimensional and robotic arrays seek to improve accuracy by reducing effects of skull aberrations and enabling longer recording sessions, respectively	Continuous perioperative cerebral blood flow velocity monitoring; emboli detection; vasodilator challenge; simultaneous vascular structure and flow visualization (duplex)	Direct measure of intracranial physiology at bedside; emboli detection	Operator-dependent; regional measure; insonation window and maintaining a stable signal can present challenges
Computed tomography					
Xenon-enhanced computed tomography[30]	Intermittent neuroimaging	Augments conventional computed tomography through inhalation of xenon by patient during image acquisition; xenon accumulates within cerebral tissue, is visible on computed tomography; detecting time to xenon enhancement and differential uptake of xenon within brain tissues allows for estimation of blood flow	Pre- and postoperative assessment of regional cerebral blood flow; preoperative vasodilator challenge	High spatial and temporal resolution for regional blood flow through brain tissue	Radiation exposure, complicated by inhaled contrast agent; cannot be used in real time at the bedside
Nuclear medicine					
Positron emission tomography[31]	Intermittent neuroimaging	The uptake of tracer-labeled water ([^{15}O]-H_2O) can be visualized with positron emission tomography to estimate the rate of blood flow through the brain; tagged glucose may reveal regional changes in metabolic activity	Pre- and postoperative assessment of regional cerebral blood flow; preoperative vasodilator challenge	High spatial and temporal resolution for regional blood flow through brain tissue	Radiation exposure; complicated by inhaled contrast agent
Magnetic resonance imaging					

(*Continued*)

Table 1. (Continued)

Imaging Modality	Category	Mechanism	Applications	Advantages	Disadvantages
Dynamic susceptibility contrast[32]	Intermittent neuroimaging	Utilizes contrast agent to estimate blood flow based on transit time through brain	Pre- and postoperative assessment of cerebral blood flow	High spatial and temporal resolution for modeling regional blood flow throughout brain; no radiation exposure	Requires intravenous contrast; constraints of magnetic resonance imaging (time, mobility, bore size); cannot use intraoperatively
Arterial spin-labeling[32]	Intermittent neuroimaging	Labels water within blood to form an endogenous tracer; comparison of control and labeled snapshot allows for inference of regional blood-flow throughout the brain	Pre- and postoperative assessment of cerebral blood flow	No contrast required; high spatial and temporal resolution; can model angiogram and parenchymal perfusion	Constraints of magnetic resonance imaging (time, mobility, bore size, portability); cannot use intraoperatively
Diffusion-weighted imaging[33]	Intermittent neuroimaging	Measures spatial differences in water anisotropy caused by restriction of water diffusion within different tissue compartments	Early detection of stroke postoperatively	Definitive standard for detection of postoperative covert stroke; no contrast required	Constraints of magnetic resonance imaging (time, mobility, bore size, portability); cannot use intraoperatively
Diffusion tensor imaging[34,35]	Intermittent neuroimaging	Incorporates multiple diffusion-weighted images taken in three perpendicular planes to create a three-dimensional model of approximate direction of fiber tracks through brain	Detection of changes in structural brain connectivity postoperatively	Offers structural basis to functional connectivity studies; can be augmented with more directions for improved resolution of fibers	Constraints of magnetic resonance imaging (time, mobility, bore size, portability); poorer resolution than other magnetic resonance imaging protocols; cannot use intraoperatively; more time-intensive than other magnetic resonance imaging methods
Diffusion kurtosis imaging[36]	Intermittent neuroimaging	Utilizes even more directions than diffusion tensor imaging, multiple magnetic field strengths to offer further insight into underlying tissue architecture	Detection of subtle changes in brain tissue architecture postoperatively	Potential to offer novel insight into changes in underlying tissue architecture	Experimental; constraints of magnetic resonance imaging (time, mobility, bore size, portability); cannot use intraoperatively; more time-intensive than other magnetic resonance imaging methods
Blood-oxygen level dependent imaging[37,38]	Intermittent neuroimaging	Forms the basis of most functional magnetic resonance imaging studies, showing neuronal activation within the brain with high spatial and temporal resolution; detects increase in oxyhemoglobin, which follows local increases in demand for oxygen by neurons (neurovascular coupling)	Pre- and postoperative characterization of brain connectome (functional connectivity)	Definitive standard for functional neuroimaging studies; exquisite spatial and temporal resolution	Constraints of magnetic resonance imaging (time, mobility, bore size, portability); cannot use intraoperatively; more time-intensive than other magnetic resonance imaging methods

The obvious caveat of this modality is the inability to discern underlying tissue architecture, which includes a mix of venous, arterial, and capillary blood and a variation of light scattering. Consequently, regional cerebral hemoglobin oxygen saturation values are typically much lower than arterial or pulse oximetry–derived values and higher than jugular bulb venous oxygenation when measured with an invasive catheter. Methods such as time and frequency domain spectroscopy report additional tissue characteristics and have been developed to overcome some of these constraints.[24] Jugular venous oximetry is an obvious comparator to near-infrared spectroscopy offering direct measurement of oxygen concentration in cerebral venous blood.[39] Such a measurement affords insight into cerebral oxygen extraction fraction but requires invasive insertion of a central cannula, and reflects oxygen extraction approaching a global/hemispheric cerebral level.[40] Near-infrared spectroscopy contrasts to this being noninvasive and capable of measurements over multiple regions, or even reconstruction into images.

There are multiple near-infrared spectroscopy techniques available to clinicians. Each technique differs with regards to the specific wavelengths used, the number of wavelengths, the number of light detectors and their distance from the emitter, and the type of modulation of emitted light (frequency and time domains).[41] These variations were developed to address two of the main disadvantages of near-infrared spectroscopy: the contamination of the signal by extracerebral tissue and inhomogeneity of light scattering within cerebral tissue.[42] Spatially resolved spectroscopy is commonly employed in clinical devices and typically comprises a single light source (two to five wavelengths) and two distal detectors. Oxyhemoglobin and deoxyhemoglobin concentrations are obtained, and regional cerebral hemoglobin oxygen saturation is calculated by the gradient of light attenuation measured between detectors, which also serves to attenuate (albeit not abolish) contamination of the signal by extracerebral tissues (fig. 1). This technique assumes that scattering properties are static and homogenous and conform to a population average.

Frequency-domain near-infrared spectroscopy and time-resolved near-infrared spectroscopy are advanced techniques that characterize both absorption and scattering. Frequency-domain near-infrared spectroscopy employs a pulsed high-frequency light source, with distortion of the frequency of these pulses at the detector related to the scattering properties of tissues. Time-resolved spectroscopy introduces a picosecond light pulse into tissue and measures the temporal point spread function of photon arrival time at a distal detector.[42] This function can be fitted to the Patterson equation, an approximation of light diffusion, to calculate absorption and scattering coefficients and thus absolute regional cerebral hemoglobin oxygen saturation.[42]

While hemoglobin species can be resolved using as few as two wavelengths of light, multiple wavelengths can be combined (in broadband spectroscopy) to resolve other tissue chromophores. Cytochrome c oxidase (complex IV in the mitochondrial respiratory chain) is of particular interest, as it reflects cellular oxygenation and metabolism.[43] The redox status of the copper complex within the cytochrome c oxidase molecular structure can be identified (and separated from hemoglobin) using broadband near-infrared spectroscopy and an adjusted Beer–Lambert technique between 780 and 900 nm.

Diffuse Correlation Spectroscopy

In addition to assessing the amount of light that is absorbed by a tissue, the degree of scattering can be measured to identify other tissue properties with similar optode detector arrangement. Diffuse correlation spectroscopy introduces a long coherence laser light source to observe small changes in light intensity that relate to scattering from movement of red blood cells.[44] An autocorrelation function of the measured fluctuations can be converted into a blood flow index and provide an estimate of regional cerebral blood flow. In contrast to positron emission tomography and magnetic resonance imaging, diffuse correlation spectroscopy offers real-time assessments of regional blood flow and has been shown to strongly correlate with cerebral blood flow values measured using transcranial Doppler ultrasound, xenon-enhanced computed tomography, and arterial spin labeling magnetic resonance imaging.[45–] This assessment can be augmented with indocyanine green dye, which can calibrate the diffuse correlation spectroscopy to absolute cerebral blood flow values.[48] The volume of tissue that is assessed is subject to the same limitations as near-infrared spectroscopy, reflecting intracranial and extracranial components; however, recent developments, such as time domain diffuse correlation spectroscopy, can be used to minimize extracerebral contamination.[25] Other approaches, including an epidural diffuse correlation spectroscopy optode to measure spinal cord blood flow, have been described to evaluate deep tissues.[26]

Scattering of light can also be induced using ultrasound directed at cerebral tissue, known as the acousto-optic effect.[27] By tagging near-infrared light using ultrasound, users can make a depth-resolved estimate of both blood flow and oxygenation.[28]

Optical Image Reconstruction Techniques

Diffuse optical tomography uses large arrays of near-infrared spectroscopy light sources and detectors, using either continuous-wave, time-resolved spectroscopy, or even diffuse correlation spectroscopy to create topographical maps of measurements. Diffuse optical tomography can predict changes in brain oxygenation and cerebral blood flow in regional maps, rather than in a single channel. This approach is used as a functional neuroimaging modality in research and can monitor brain physiology in real time.[26]

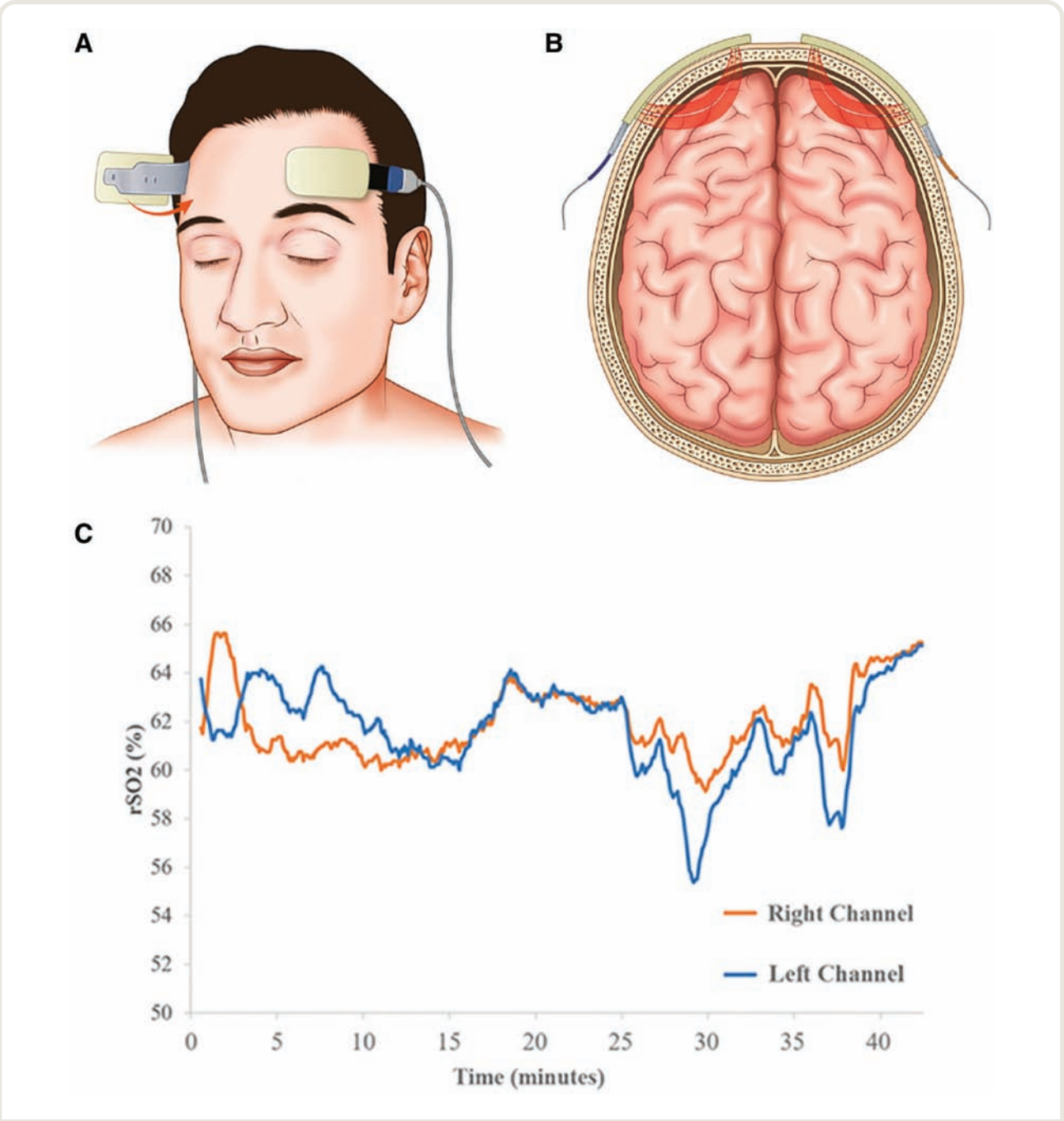

Fig. 1. (*A*) Placement of near-infrared spectroscopy optodes in the bifrontal configuration. (*B*) Cross-section of the head depicting the light paths from two emitters to the detector of the near-infrared spectroscopy optodes. (*C*) Regional cerebral hemoglobin oxygen saturation waveforms acquired bilaterally from the two near-infrared spectroscopy optodes.

Advances in system miniaturization and analysis software are increasing the potential usefulness of diffuse optical tomography in the perioperative setting.[27]

Ultrasonographic Techniques

Transcranial Doppler ultrasound is a well-established technique for evaluating cerebral perfusion intraoperatively. It uses Doppler ultrasound (2 MHz) to insonate basal cerebral vessels. Doppler ultrasound reports a flow velocity, which allows for the detection of emboli through characteristic features on the frequency spectrogram. Further ultrasonographic iterations include color M-mode, which reports velocity and depth; B-mode color Doppler, which reports a two-dimensional image and Doppler flow; and

three-dimensional Doppler. The Doppler frequency shift between emitted and received ultrasound relates to the speed of blood flow in the region. Assuming a constant vessel radius (one theoretical limitation to this technique), the measured changes in flow velocity are proportional to cerebral blood flow.[41] This technique relies on identifying a suitable transcranial window, which is an area where acoustic signal can move adequately through tissue and insonation of the vessels of interest can be achieved. Transcranial Doppler ultrasound is frequently performed over the temporal bone, which enables the assessment of the middle, anterior, and posterior cerebral arteries (fig. 2A). Insonation of other major vessels is achievable using submandibular, transorbital, or suboccipital probe placements (fig. 2B). Transcranial Doppler ultrasound measures flow velocity through a given vessel in centimeters per second (fig. 2C).[29] Transcranial Doppler ultrasound is a powerful clinical tool, but its use is associated with several theoretical limitations. First, prolonged monitoring requires a trained and experienced transcranial Doppler ultrasound operator due to some challenges with maintaining probe position and window visualization even with the aid of commercially available fixation devices. Second, due to artefacts created from diathermy and movement intraoperatively, it can be difficult to maintain insonation for the continuous measurement of flow and identification of markers of dysfunction or injury. Vessel identification using concurrent B-mode ultrasound and tracking using robotic transcranial Doppler ultrasound might assist with this challenge.

Magnetic Resonance Imaging Techniques

Magnetic resonance imaging can be used to diagnose perioperative stroke in the early stages and, due to its exquisite spatial resolution, is excellent for assessing regional blood flow in the brain.[32] While structural magnetic resonance imaging scans can discern tissue types based on the intrinsic constraints on hydrogen atoms within tissue, advanced sequences allow for identification and measurement of regions of the brain where hydrogen atoms within blood are flowing. The two main imaging sequences for acquisition of cerebral blood flow data using magnetic resonance imaging are dynamic-susceptibility contrast perfusion and arterial spin-labeling perfusion. Dynamic-susceptibility contrast perfusion depends on administering a contrast agent, with the transit time of a bolus of known volume through cerebral tissue providing an estimate of cerebral blood flow, whereas arterial spin-labeling perfusion measures the transit time of magnetically labeled water in blood.[32] Both magnetic resonance imaging and positron emission tomography cerebral blood flow measurements offer spatial information regarding cerebral perfusion, such that regional comparisons of cerebral blood flow can be obtained. Magnetic resonance imaging can also be used to assess flow through large vessels in the form of phase-contrast magnetic resonance imaging, which provides an estimate of flow velocity.[31] While magnetic resonance imaging has tremendous utility in the clinic, an important drawback in the perioperative setting is the limited scope to acquire real-time point-of-care information.

Imaging Metrics

Cerebral Blood Flow

Cerebral blood flow can be assessed globally in the large basal cerebral blood vessels or regionally in the microcirculation using the techniques described in the Perioperative Neuroimaging and Neuromonitoring section. Combining measures of cerebral blood flow with blood pressure or oxygenation data allow for the evaluation of autoregulation and cerebral metabolic rate, respectively. Functional connectivity between neuronal networks can be measured by correlating simultaneous monitoring of cerebral blood flow across multiple brain regions.

Cerebral Metabolic Rate of Oxygen

The cerebral metabolic rate of oxygen can be derived using a Fick model by measuring cerebral blood flow and the oxygen saturation difference between arterial and venous blood.[52] Cerebral metabolic rate of oxygen is an estimate of the metabolic demands of cerebral tissue and remains relatively constant during physiologic conditions. As cerebral metabolic rate of oxygen is the amount of oxygen extracted by tissue, it depends on both cerebral blood flow and the oxygen extraction fraction. An assessment of cerebral metabolic rate of oxygen in the perioperative period can indicate whether cerebral tissue is being optimally perfused.

Cerebral metabolic rate of oxygen can be assessed at the bedside using time-domain near-infrared spectroscopy concurrently with transcranial Doppler ultrasound or diffuse correlation spectroscopy[53] *via* a Fick model. This utility provides clinicians with the ability to monitor cerebral metabolic rate of oxygen and hemodynamic adequacy in the intraoperative period. Of note, however, near-infrared spectroscopy limits assessments to a small region of the cerebral cortex, which might not accurately reflect whole-brain cerebral metabolic rate of oxygen and is not a direct measure of oxygen consumption.

Accurate estimates of global cerebral metabolic rate of oxygen can be obtained using magnetic resonance imaging. Two emerging methods for assessing cerebral metabolic rate of oxygen using magnetic resonance imaging are T2 relaxation under spin tagging and susceptometry-based oximetry. T2 relaxation under spin tagging, which uses the same spin-labeling technique as arterial spin-labeling perfusion, can be used to determine the oxygen content of venous blood within the venous sinuses of the cranium and estimate deoxyhemoglobin concentration based on the T2 relaxation time.[54] In contrast, susceptometry-based oximetry determines the magnetic susceptibility of blood within the venous sinuses to estimate deoxyhemoglobin concentration.[55] It is possible to estimate cerebral metabolic rate of

oxygen by pairing either T2 relaxation under spin tagging or susceptometry-based oximetry sequences with flow estimates from phase-contrast magnetic resonance imaging.[56]

Cytochrome c Oxidase Imaging

In addition to assessing deoxyhemoglobin and oxyhemoglobin, there is growing interest in imaging other chromophores related to cerebral oxygenation by way of broadband near-infrared spectroscopy. One of these chromophores is cytochrome c oxidase, which is the terminal electron acceptor in the mitochondrial electron transport chain. Previous studies have shown that cytochrome c oxidase concentrations increase when oxygen levels in cerebral tissue are elevated and decrease during periods of hypoxia–ischemia.[57] Changes in oxidized cytochrome c oxidase concentration convey unique benefits over deoxyhemoglobin and oxyhemoglobin, as these changes are more specific for cerebral metabolism and less susceptible to contamination by extracranial tissue.[58] The change in oxidized cytochrome c oxidase concentration index has been validated in both animal models and healthy adults.[57,59,60]

Cerebral Blood Flow Autoregulation and Vasodilatory Reserve

Cerebral autoregulation is the mechanism by which blood vessels in the brain dilate and constrict during fluctuations in perfusion pressure to adjust cerebrovascular resistance to maintain a constant cerebral blood flow. Autoregulation functions over a range of perfusion pressures, outside of which these vascular mechanisms fail, and tissue becomes susceptible to injury.[61,62]

Cerebral autoregulation is often conceptualized in terms of a static model. Within this model, blood flow remains stable when the mean arterial pressure (MAP) is between 50 and 150 mm Hg; blood pressure within this range results in stable cerebral blood flow,[63] whereas pressure below this range leads to hypoperfusion, and pressure over this range leads to hyperperfusion. Static autoregulation analysis is only possible when a range of MAP values have either been induced or have occurred physiologically so that consequent cerebral blood flow changes can be identified at a steady state (fig. 3). Ideally, modeling this curve for each patient would enable identification of the upper and lower limits of blood pressure beyond which cerebral blood flow is variable.

In contrast to static models, dynamic models of autoregulation incorporate the temporal component of vascular reactivity to enable less invasive and more rapidly calculable indices of autoregulation. Rapid changes in cerebral perfusion pressure invariably result in changes in cerebral blood flow until the cerebral vasculature can adequately adjust to the altered conditions, typically within 5 to 15 s.[62] However, gradual changes to cerebral perfusion pressure are more readily compensated for by the vasculature and should not lead to detectable changes in cerebral blood flow.

The main method for assessing cerebrovascular autoregulation involves simultaneously monitoring arterial blood pressure or a surrogate for cerebral blood flow, for example *via* transcranial Doppler ultrasound–measured flow velocity, near-infrared spectroscopy monitored regional cerebral hemoglobin oxygen saturation, or invasively monitored brain tissue oxygenation. Autoregulation acts as a high-pass filter in the relationship between arterial blood pressure and cerebral blood flow, and its effectiveness can be defined by the phase and strength of the relationship (known as transfer function). Continuous monitoring is desirable clinically but can be challenging to conduct over long periods with ultrasonography due to the window, probe fixation, and sources of artefact. Other surrogates for cerebral blood volume and cerebral blood flow can be used in a similar fashion. Most notably, intracranial pressure (ICP) has been used as a surrogate for the assessment of cerebral blood volume and/or cerebrovascular reactivity. Although not used electively in the perioperative setting, this signal processing approach has been generalized to other modalities. When autoregulation is intact, slow waves (0.003 to 0.05 Hz) in ICP and MAP are not correlated, which suggests that slow changes in perfusion pressure are being adequately accommodated.[64] When autoregulation is compromised, these slow waves are correlated, indicating that local ICP is coupled to MAP without compensating vascular mechanisms. This relationship is called the pressure reactivity index, which is the Pearson correlation coefficient between MAP and ICP values.[65] Pressure reactivity index values range from –1 to 1, with negative and lower values indicating better performance of autoregulatory mechanisms. Pressure reactivity index values greater than 0.3 indicate that autoregulation is not intact.

After obtaining multiple ICP and MAP measurements, a curve can be constructed that demonstrates the relationship between cerebral perfusion pressure and pressure reactivity index, as shown in figure 4. This curve is U-shaped, with the theoretically optimal cerebral perfusion pressure values resulting in the lowest possible pressure reactivity index values, and pressure reactivity index values increasing as cerebral perfusion pressure deviates from the range of optimal values (reflecting the cerebral perfusion pressure range of cerebral autoregulation).[66] As such, repeated calculation of pressure reactivity index at different cerebral perfusion pressure values allows estimation of the optimum cerebral perfusion pressure, and instantaneous determination of pressure reactivity index at a specific cerebral perfusion pressure may indicate whether the upper or lower limit of autoregulation has been surpassed.

While invasive monitoring of ICP is not feasible during routine noncardiac surgery, similar metrics have been developed for neuroimaging. For transcranial Doppler ultrasound, simultaneously measuring middle cerebral artery flow velocity and systemic arterial blood pressure permits determination of the mean velocity index, with Pearson correlation coefficient assessing slow waves in ICP and arterial blood pressure waveforms. With near-infrared

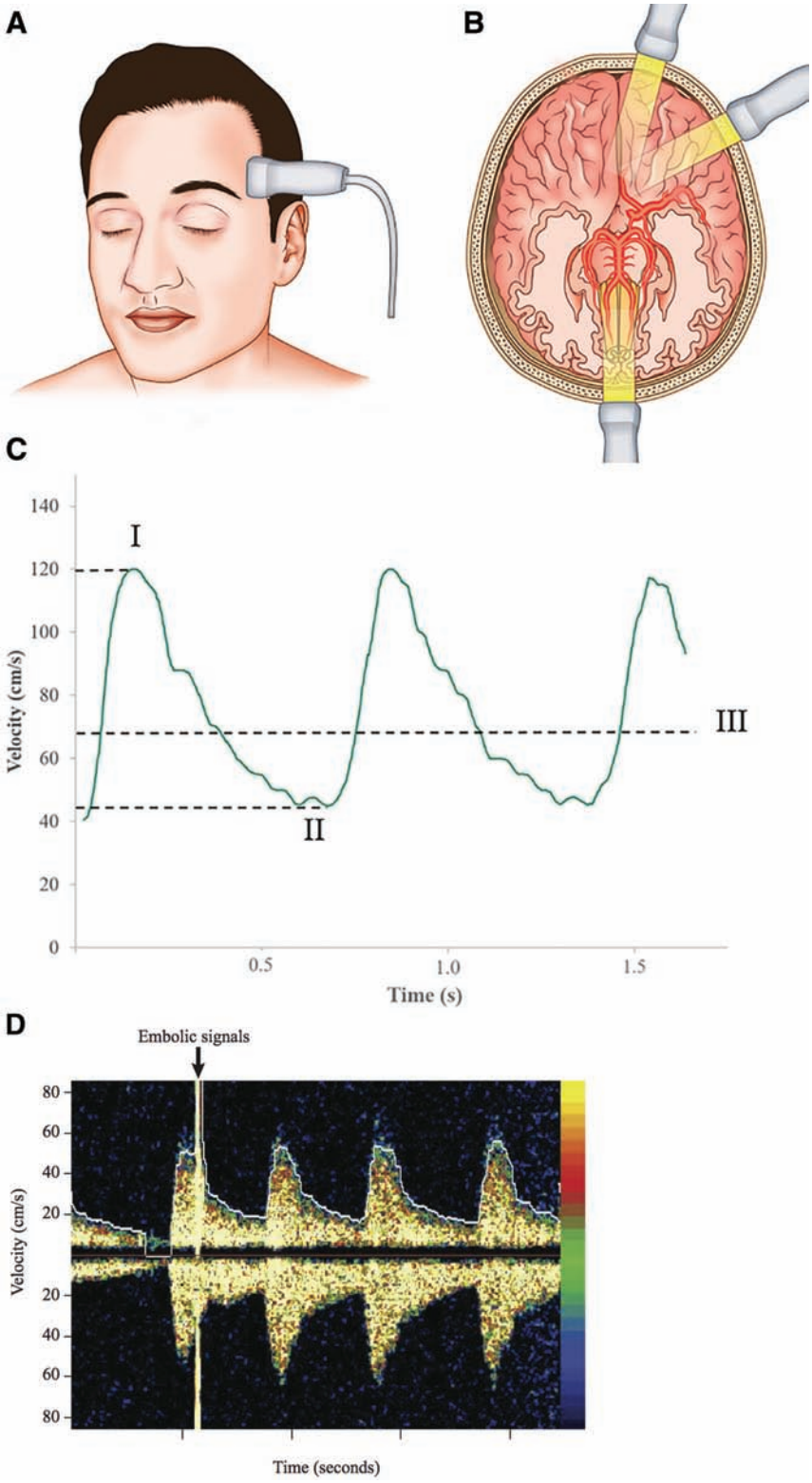

Fig. 2. (*A*) Transtemporal placement of a transcranial Doppler transducer. (*B*) Cross-section of the skull depicting insonation of the basal cerebral arteries. (*C*) Simplified waveform of flow velocity through the middle cerebral artery obtained by transcranial Doppler transducer depicting (I) peak systolic velocity, (II) end-diastolic volume, and (III) mean flow velocity. (*D*) Actual transcranial Doppler transducer waveforms collected intraoperatively while transducing the middle cerebral artery.

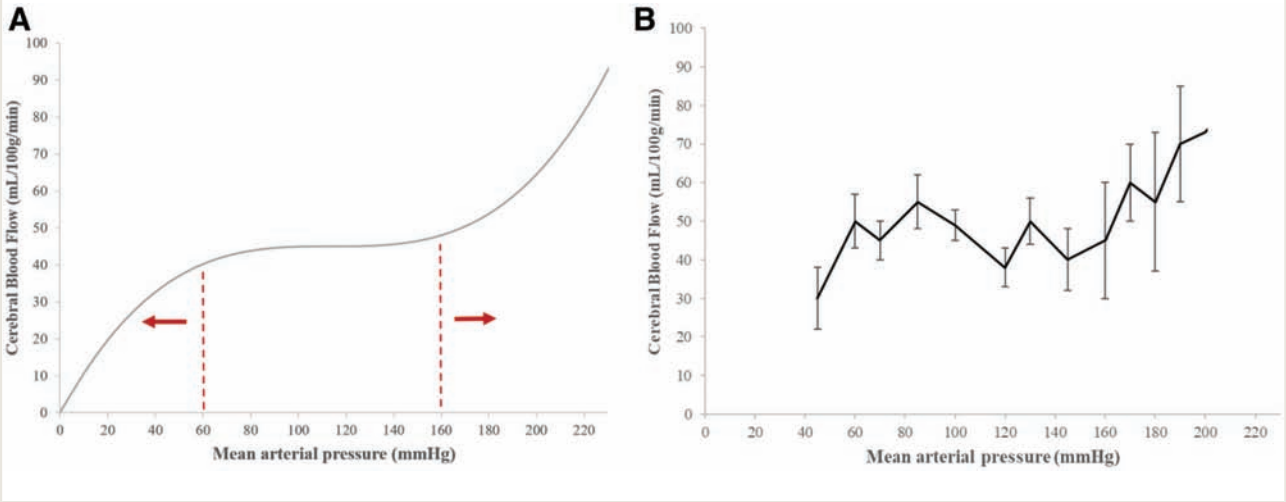

Fig. 3. (*A*) A stylized graph of the relationship between cerebral blood flow and mean arterial pressure is linear beyond the upper and lower limits of autoregulation (indicated by the *red dashed lines* and *arrows*). Between the upper and lower limits, autoregulation compensates for increases or decreases in mean arterial pressure to maintain a constant cerebral blood flow. (*B*) Real-life data, identifying the limits of autoregulation through static assessment is not as straightforward.

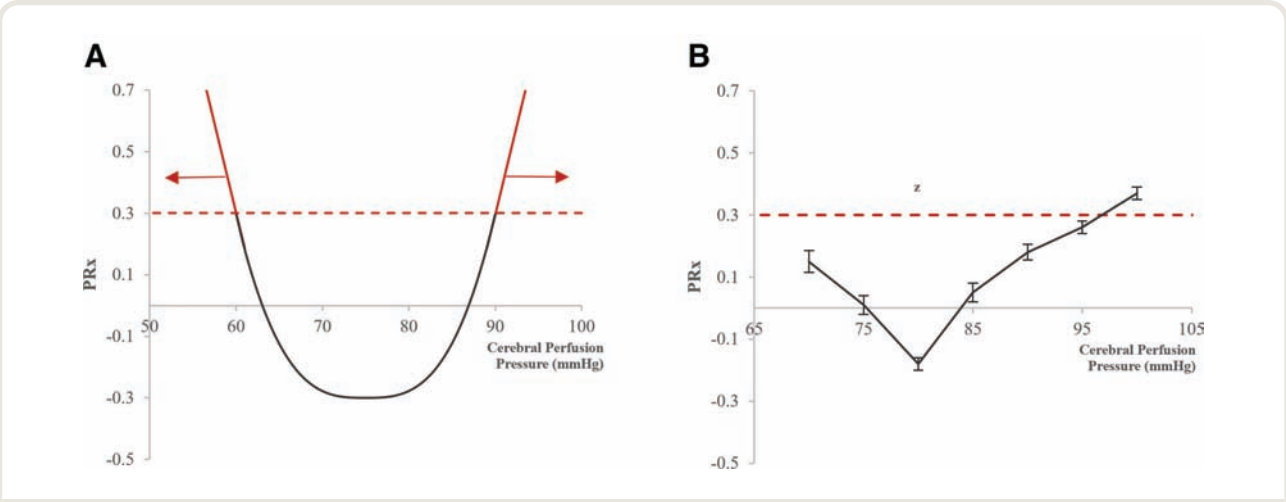

Fig. 4. (*A*) Theoretical U-shaped curve of pressure reactivity index as a function of cerebral perfusion pressure. The *red dashed line* depicts the widely used cutoff value of "safe" pressure reactivity index: 0.3. cerebral perfusion pressure values corresponding to pressure reactivity index values greater than 0.3 are insufficient for maintaining functional cerebral autoregulation (*red arrows*). (*B*) Pressure reactivity index plot acquired from a patient in real time in an intensive care setting.

spectroscopy, the tissue oxygen index is derived from slow waves in regional cerebral hemoglobin oxygen saturation and arterial blood pressure waveforms, and the total hemoglobin index is derived from total hemoglobin and arterial blood pressure.[67] As diffuse correlation spectroscopy provides a direct estimate of cerebral blood flow, it has the potential to form the basis for a more sensitive autoregulation index than near-infrared spectroscopy.[68]

Advances in signal processing promise to improve on the fidelity of these noninvasive techniques. For example, wavelet analysis can be used to define changes in frequency and time domains simultaneously and has the potential to provide more accurate determinations of optimum cerebral perfusion pressure (fig. 5).[69]

An additional method for assessing autoregulation is to determine vasodilatory reserve. Vasodilatory reserve assesses the maximal capacity of a patient's cerebral vasculature to dilate to accommodate decreases in blood pressure.[30] This assessment can be made preoperatively through a vasodilatory challenge using carbon dioxide or acetazolamide as

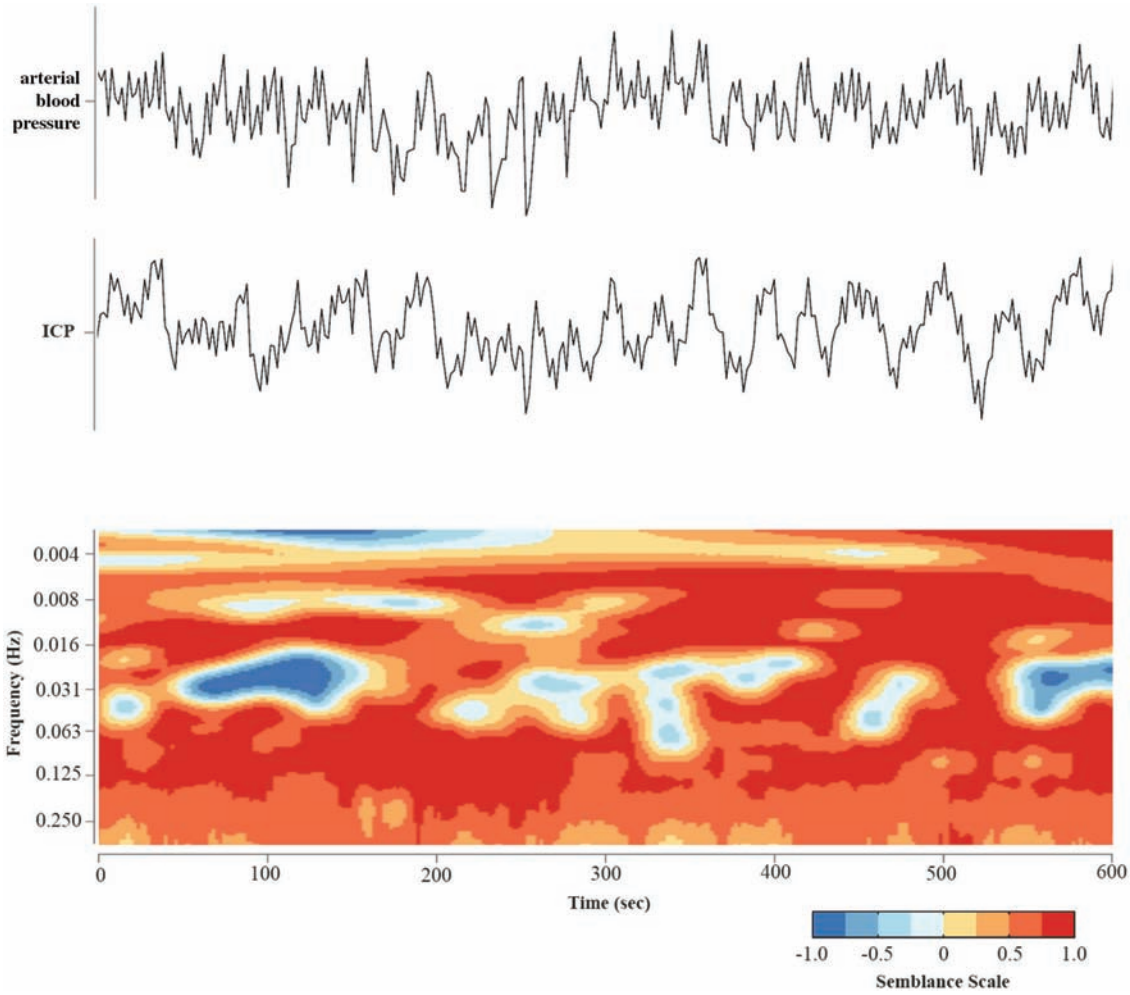

Fig. 5. Wavelet semblance analysis reflecting instantaneous phase differences between arterial blood pressure and intracranial pressure (ICP) in a patient with traumatic brain injury.[61] Visual inspection of the arterial blood pressure and ICP waveforms (*top*) suggests that they are largely in phase, which is reflected in the semblance analysis (*bottom*). High semblance indicates that changes in arterial blood pressure are in phase with changes in ICP (*i.e.*, a failure of independent control of blood flow within the brain reflects pressure passive changes in ICP). Physiologically, high semblance at shorter wavelengths (sudden changes in arterial blood pressure) is less indicative of autoregulation failure. Autoregulation acts like a high-pass filter, passing higher frequency waves and dampening lower frequency.

measured with computed tomography or magnetic resonance imaging.[70–72] Critical closing pressure, which is the cerebral perfusion pressure where measurements of flow are modeled to drop to zero, can be derived from transcranial Doppler ultrasound or diffuse correlation spectroscopy.[46]

Connectomics and Brain Mapping

The brain achieves its function by coordinated action between millions of neuronal networks. Connectomics is the study of brain connectivity between these networks, and involves the analysis of synchronous activity within the brain as detected by changes in regional blood flow or through imaging of underlying tissue architecture (fig. 6).[22] The pattern of neural activity in different regions of the brain under a given state is referred to as a *connectome*.[73] This technique reveals the underlying structural and functional connectivity of the brain, which differs between healthy and disease states and might offer novel avenues for detecting subclinical or overt injuries early in their development.

Functional magnetic resonance imaging has become the definitive standard for assessing functional brain connectivity.[37] Blood oxygenation level–dependent contrast-enhanced imaging is the predominant functional magnetic resonance imaging method. Local increases in metabolic activity result in transient accumulation of paramagnetic deoxyhemoglobin, but because of neurovascular coupling, there is a reflex increase in cerebral blood flow, leading to rapid clearance of deoxyhemoglobin. This decrease in deoxyhemoglobin concentration produces a blood

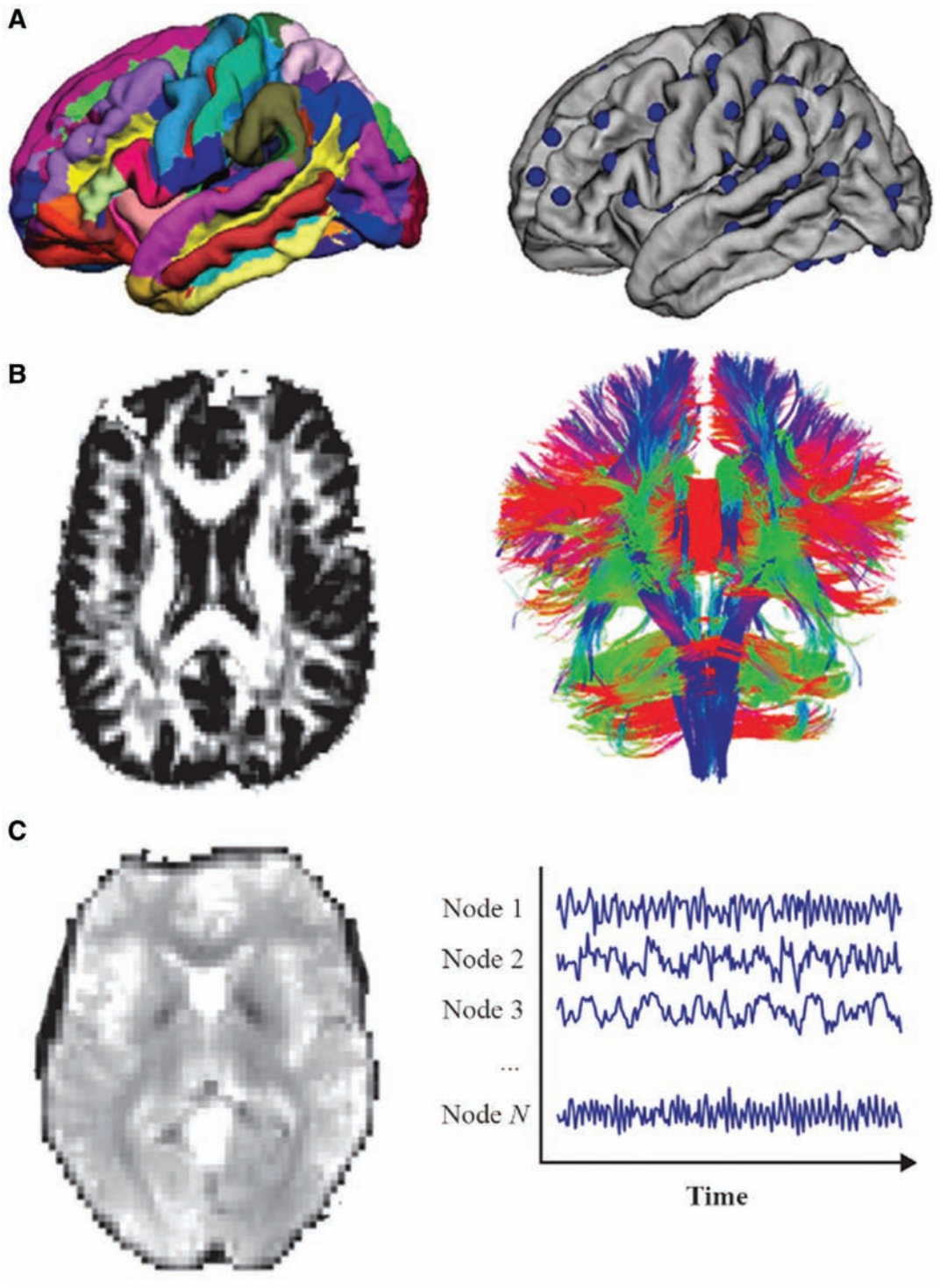

Fig. 6. (*A*) The brain is divided into regions or nodes, which form the basis for mapping connectivity. These regions can be defined anatomically (*left*) or functionally (*right*). (*B*) Structural connectivity can be assessed with diffusion tensor imaging (*left*) or more advanced diffusion magnetic resonance imaging sequences for modeling white matter tracts. These tractograms (*right*) can model the presence and strength of connections between predefined brain regions (the colors correspond to the direction of the tracts in x-y-z planes). (*C*) Functional connectivity is determined using brain oxygen level–dependent magnetic resonance imaging (*left*). Simultaneous assessment of activity within different nodes (*right*) allows evaluation of connectivity. In the displayed graph, nodes 2 and 3 are in phase and likely connected, whereas node 1 does not exhibit activity synchronous with the other two nodes. Node *N* shows synchronous activity with all of the above nodes.[22]

oxygenation level–dependent signal and acts as a proxy for neurologic activation.[38] Regional activation patterns in the cerebral cortex are analyzed topologically, allowing networks of interconnected regions to be identified; these networks similarly connect to one another, adding layers of complexity to brain connectivity.[73]

Connectivity can also be assessed structurally by generating tractograms using diffusion magnetic resonance imaging.[34] Diffusion-weighted magnetic resonance imaging detects restriction of diffusion by cellular compartments within the brain. Diffusion-weighted magnetic resonance imaging will detect an increased signal where water is more tightly restricted, whereas it will detect a decreased signal where water is diffusing more freely. The signal received is related to the isotropy of a volume of water (anisotropy is a term indicating that diffusion is restricted).[74] Tractography methods, such as diffusion tensor imaging, take advantage of the strong anisotropy of white matter tracts to generate measurements reflecting axonal connections between brain regions.[74]

The most basic diffusion magnetic resonance imaging tractography technique is diffusion tensor imaging, which uses six diffusion directions acquired in three perpendicular axes to derive tracts from mathematical tensors.[35] More robust tractograms can be derived though high-angular resolution diffusion imaging, which involves the acquisition of diffusion images in many directions (often hundreds). This approach allows for the construction of complex orientation distribution functions that provide additional information regarding fiber orientation and density.[75,76]

Further information regarding the composition of tissue can be acquired using diffusion kurtosis imaging. This technique involves the acquisition of images at multiple magnetic field strengths to determine how diffusion of water in a given region of the brain differs from a normal Gaussian distribution[36,77] (fig. 7). Due to its high sensitivity, diffusion kurtosis imaging can also be used to identify alterations to the cellular and parenchymal architecture. This utility means diffusion kurtosis imaging can be used in the diagnosis of neurodegenerative conditions such as Parkinson disease and Alzheimer disease.[78,79]

While magnetic resonance imaging is useful in the preoperative and postoperative settings, it is only used intraoperatively during neurosurgery. Near-infrared spectroscopy is a viable alternative to magnetic resonance imaging as it can achieve a similar level of functional mapping. Functional near-infrared spectroscopy is a form of cerebral oximetry that uses multiple optodes arranged in a wearable cap to capture measurements of cerebral oxygenation across the entire cortex, instead of just a sample of tissue (as in bifrontal near-infrared spectroscopy).[80] Signals from these optodes can be modeled through diffuse optical tomography,[27] which allows for oxyhemoglobin and deoxyhemoglobin concentrations to be mapped to the cerebral cortex with high spatial and temporal resolution.

Clinical Applications

Neuroimaging for Preoperative Assessment

Clinicians have access to a myriad of tools to help them estimate the risk of overt, clinically apparent surgical complications based on demography and clinical factors. There is, however, a lack of tools available for the practical individualized assessment and estimation of subtle neurologic impairment.[81,82] Advances in technology have the potential to optimize current preoperative assessment practices and further minimize the risk of surgical complications. One potential strategy that could benefit patient outcomes and improve resource allocation is to use neuroimaging to conduct a detailed preoperative assessment of brain frailty.

Neuroimaging methods have achieved mainstream utility in the context of neurosurgery for functional and structural brain mapping (discussed in more detail in the Postoperative Diagnostic Imaging Assessment section).[83] Outside of neurosurgery, however, little evidence exists for the usefulness of preoperative neuroimaging methods; consequently, they are rarely used.[84] There has been growing focus on objective quantification of patient vulnerability beyond basic features pertaining to medical history and demographics, including the use of preoperative assessments of cognitive function, which enable risk stratification and specific measurement of cognitive decline. This practice is particularly true for the assessment of cognitive vulnerability, a major issue in older patients who have a high risk of postoperative cognitive decline and delirium.[85,86] This section highlights specific applications of neuroimaging in the preoperative period toward this same end.

Vasodilatory Reserve

Preoperative assessment of vasodilatory reserve aims to assess the need for surgical intervention for conditions involving the major blood vessels of the head and neck, as well as enabling risk stratification, such as in moyamoya disease.[87] For carotid endarterectomy, preoperative assessment of the vasodilatory reserve of the cerebral vasculature has been shown to aid in predicting adverse cerebrovascular events.[88] Carotid endarterectomy is associated with a high risk of transient hypoperfusion and embolization, as well as the possibility of cranial hyperperfusion syndrome immediately after correction of the stenosis.[89] As such, vasodilatory reserve is essential for maintaining adequate cerebral perfusion during and immediately after this surgery.

Across several small clinical studies, impaired vasodilatory reserve has been associated with an increased risk of postoperative stroke or transient ischemic attack post–carotid endarterectomy.[30,71,72] Several studies used transcranial Doppler ultrasound or 133-xenon emission tomography to monitor blood flow before and after the administration of acetazolamide (a potent vasodilator). An increase in middle cerebral artery flow velocity of 40% or more, as measured by

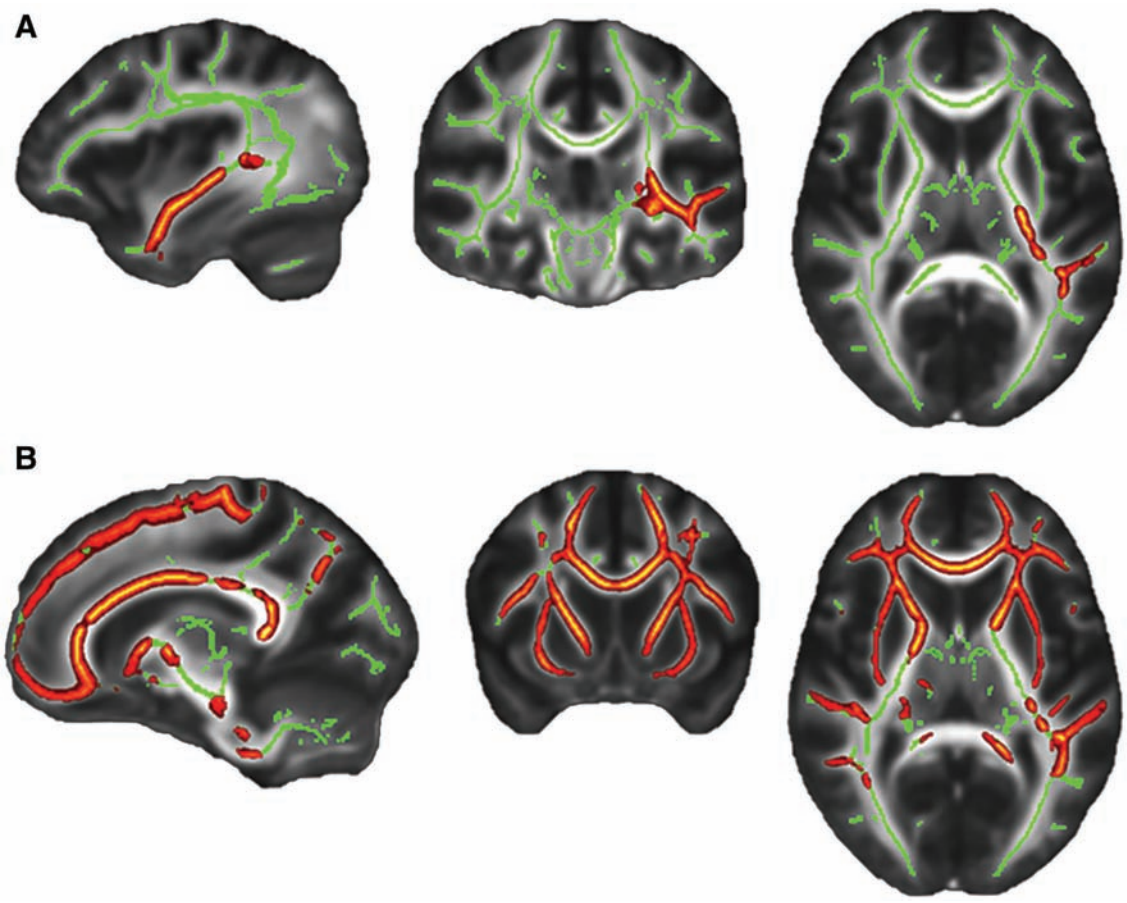

Fig. 7. Results of tract-based analysis of healthy adult brains. The fractional anisotropy skeleton, which depicts underlying white matter tracts, is shown in *green*. The *red* regions depict areas where diffusion kurtosis scalars were significantly associated with (*A*) emotional memory and (*B*) emotional bias. Increased diffusivity and reduced cellular complexity within these regions of white matter were associated with emotional dysregulation.[75]

transcranial Doppler ultrasound, was found to be protective against postoperative cerebrovascular events, such as stroke or transient ischemic attack, whereas an absent or less than 40% increase in cerebral blood flow was associated with an increased risk.[30] Similarly, an increase in cerebral blood flow of more than 20% after inspiration of 8% CO_2 was associated with a decreased risk of cerebrovascular events.[72] In a meta-analysis of 991 patients with carotid stenosis or occlusion, reduced vasodilatory reserve was associated with a fourfold increased risk of stroke or transient ischemic attack.[64]

While these studies indicate that preoperative assessment of cerebrovascular function, specifically vasodilatory reserve, might aid in predicting injury associated with carotid endarterectomy, no studies have validated the use of preoperative neuroimaging for risk assessment in patients undergoing other procedures. An assessment of indices of cerebrovascular function before noncardiac surgery is an enticing avenue of future research. This approach could be used as a marker of autoregulation, aid with the prediction of the optimal perioperative MAP target, and improve patient risk stratification. Such markers could guide strategies for monitoring, intraoperative management, and postoperative diagnostic testing, although data are lacking to support these applications.

Cerebral Oxygen Saturation

In the context of major cardiac surgery, one study assessed the utility of preoperative regional cerebral hemoglobin oxygen saturation assessment. In 1,178 patients undergoing procedures requiring cardiopulmonary bypass, a minimum preoperative absolute regional cerebral hemoglobin oxygen saturation score recorded by bifrontal near-infrared spectroscopy of less than 50% was associated with a significantly increased risk of both mortality and morbidity.[90] However, a regional cerebral hemoglobin oxygen saturation less than 50% was also associated with a higher burden of comorbidities, including decreased left

ventricular ejection fraction and heart failure. Thus, while it is unclear whether including regional cerebral hemoglobin oxygen saturation itself in preoperative screening improves the prediction of patient risk associated with cardiac surgery, further investigation in noncardiac surgical settings is warranted.

Intraoperative Monitoring

Application of neuroimaging methods in the intraoperative period has the potential to protect patients from injury through enabling early detection of organ hypoperfusion and avoidance of tissue oxygen supply–demand mismatch. Use of available modalities are obviously limited to those that can be applied noninvasively with minimal imposition on the surgical field and are safe for continuous real-time monitoring. As such, most intraoperative applications in noncardiac, nonneurologic surgery involve the use of optical and ultrasonographic methods for monitoring cerebral perfusion.

Intraoperative monitoring of cerebral perfusion has primarily been validated for major cardiac procedures requiring cardiopulmonary bypass. In this setting, decreases in cerebral blood flow or cerebral oxygenation during the operative period are usually directly correlated with surgical or anesthetic factors (for example, large-vessel manipulation and/or clamping, pump flow alterations, blood volume loss) and are thus readily corrected. Currently, however, intraoperative neuromonitoring for noncardiac surgery is still an emerging field.

Limitations of Cerebral Oxygen Saturation Monitoring

Our understanding of the clinical relevance of cerebral regional cerebral hemoglobin oxygen saturation or hemoglobin concentrations is still incomplete in the intraoperative setting. A systematic review of the literature reported mixed findings, with insufficient evidence yet to support or refute an outcome benefit.[91] The utility of any monitoring technique relates to the effectiveness of clinical intervention. For example, interventions in response to regional cerebral hemoglobin oxygen saturation focus on increasing cerebral oxygen delivery by manipulating several physiologic processes, including blood pressure, ventilation and carbon dioxide, inspired oxygen fraction, transfusion, and reduction of neuronal activity by increasing depth of anesthesia, to reduce cerebral metabolic rate of oxygen. Many critics highlight intrinsic limitations of widely used clinical near-infrared spectroscopy monitors, including the inability to discern differences in underlying tissue composition within the sampled near-infrared spectroscopy region.[24] Therefore, the regional cerebral hemoglobin oxygen saturation value depends on not only tissue oxygenation but also tissue composition, which cannot be readily standardized between patients or tests. This point was demonstrated by Bickler *et al.*, who compared multiple oximetry devices and found high variability dependent on underlying tissue architecture.[92] As such, baseline regional cerebral hemoglobin oxygen saturation values in healthy adults vary considerably, with studies reporting average baseline regional cerebral hemoglobin oxygen saturation values between 55 and 80%. However, studies often focus on changes in cerebral oxygen saturation rather than absolute measurements. While alterations in regional cerebral hemoglobin oxygen saturation in the intraoperative period have been associated with adverse patient outcomes during some major cardiac and vascular procedures, the definitions of significant decreases in regional cerebral hemoglobin oxygen saturation have varied considerably.

It is possible that time-domain or frequency-domain near-infrared spectroscopy might reduce the effects of heterogeneity in tissue composition and allow the detection of subtle changes in oxyhemoglobin or deoxyhemoglobin concentrations. Variations in total hemoglobin content might prove to be more accurate than regional cerebral hemoglobin oxygen saturation changes for detecting intraoperative hemodynamic perturbations. Future research should focus on emerging optical imaging methods within this area to help resolve these unanswered questions.

Perioperative Delirium

Multiple studies have evaluated associations between intraoperative neuromonitoring variables and the incidence of delirium after surgery. These studies are confounded by the constraints and variability underlying near-infrared spectroscopy, which have been explained in detail, as well as evolution in the appropriate use of screening and diagnostic tools for neurologic outcome. A meta-analysis from 2020 found that intraoperative regional cerebral hemoglobin oxygen saturation monitoring was not associated with a decreased rate of postoperative delirium, as defined by standardized clinical screening tools including the Confusion Assessment Method, the Confusion Assessment Method–Intensive Care Unit, the *Diagnostic Statistical Manual of Mental Disorders* (4th or 5th edition) criteria, or the Mini Mental Status Examination.[93] Of note, the Mini Mental Status Examination is not considered an accurate diagnostic test for delirium, suggesting that some studies employ inappropriate assessment tools, and highlights a need for objective measures of postoperative neurocognitive disorders.[94]

However, some noncardiac studies have detected associations between specific near-infrared spectroscopy metrics and postoperative delirium. A 2018 study investigated the utility of near-infrared spectroscopy during urgent surgery for hip fracture in 40 patients over 65 yr of age.[95] Investigators commenced bifrontal near-infrared spectroscopy monitoring from the moment patients were admitted to the hospital, such that cerebral oxygenation was monitored during resuscitation and surgery. The results showed that lower nadir regional cerebral hemoglobin oxygen

saturation values were associated with increased risks of postoperative delirium, as well as 30-day mortality. While both studies identified statistically significant associations between regional cerebral hemoglobin oxygen saturation decreases and adverse outcomes, the reported differences were of little clinical significance, and the studies included small cohorts of high-risk patients. Thus, these findings provide limited support for the use of intraoperative regional cerebral hemoglobin oxygen saturation monitoring to reduce the likelihood of delirium after noncardiac, nonneurologic surgery.

Postoperative Cognitive Dysfunction

Recognizing the 2018 change in nomenclature to the preferred "neurocognitive dysfunction" and "neurocognitive disorder," most literature has historically reported postoperative cognitive dysfunction.[6] Postoperative cognitive dysfunction has been associated with multiple near-infrared spectroscopy and transcranial Doppler ultrasound values in several noncardiac cohorts. A meta-analysis detected a statistically significant decrease in postoperative cognitive dysfunction incidence when intraoperative regional cerebral hemoglobin oxygen saturation monitoring was used (odds ratio, 0.53 [95% CI, 0.39 to 0.73]; n = 1,380).[93] Among the studies included in this meta-analysis, postoperative cognitive dysfunction definitions were primarily based on decreased postoperative performance on the Mini Mental Status Examination or Montreal Cognitive Assessment, recognizing the perioperative limitations and time constraints of the definitive standard of neuropsychiatric assessment batteries. The efficacy of regional cerebral hemoglobin oxygen saturation monitoring for preventing postoperative cognitive dysfunction has been examined in patients undergoing cardiac surgery or major vascular surgery and major abdominal surgery.[96,97]

For major cardiac and vascular surgery, a decrease in absolute regional cerebral hemoglobin oxygen saturation to less than 50% or a decrease of more than 20% in regional cerebral hemoglobin oxygen saturation below baseline has been associated with postoperative cognitive dysfunction.[98] Additionally, an absolute regional cerebral hemoglobin oxygen saturation nadir less than 35% or a transient decrease in absolute regional cerebral hemoglobin oxygen saturation to less than 40% persisting for more than 10 min has been associated with postoperative neurologic dysfunction.[97]

In a retrospective study of 125 older adults (older than 65 yr) undergoing knee replacement surgery with spinal anesthesia, bifrontal near-infrared spectroscopy was used to monitor decreases in cerebral oxygen saturation during surgery.[99] Interhemispheric differences in regional cerebral hemoglobin oxygen saturation throughout the procedure were associated with memory decline at 3 months postoperatively.

In 122 patients undergoing major abdominal surgery, regional cerebral hemoglobin oxygen saturation–guided care was found to decrease the incidence of postoperative cognitive dysfunction when assessed by serial Mini Mental Status Examinations.[96] In a retrospective study of 46 older patients (greater than 55 yr of age) undergoing major abdominal surgery, cerebral near-infrared spectroscopy was used to assess the safety of specific aspects of surgery. A statistically significant regional cerebral hemoglobin oxygen saturation decrease (greater than or equal to 20% decrease below baseline) occurred in 11 of the 46 patients, and in 6 of these individuals, there was a clear temporal association between the decrease in regional cerebral hemoglobin oxygen saturation and intraoperative hemorrhage.[100] Decreases in regional cerebral hemoglobin oxygen saturation occurred despite systolic arterial blood pressure remaining normal and were corrected only by blood transfusion. Across the entire cohort, there was a statistically significant correlation between decreases in regional cerebral hemoglobin oxygen saturation and hemoglobin, correctable by blood transfusion, indicating that monitoring conventional hemodynamic parameters alone was insufficient for identifying periods of insufficient cerebral oxygen supply. Oximetry was necessary for real-time assessment of end-organ perfusion during periods of hemodynamic compromise. An exploratory study investigated the relationship between intraoperative factors during liver transplantation and cerebral oxygenation to identify which steps were associated with an increased risk of end-organ hypoperfusion.[101] Several procedural steps, including portal vein clamping, administration of vasoactive substances, and phlebotomy, were found to significantly alter cerebral perfusion.

Beach Chair Positioning

Near-infrared spectroscopy is commonly recommended in orthopedic operations requiring the beach chair position. Patient positioning can reduce cerebral perfusion pressure based on the relative position to the heart. This positioning can threaten cerebral perfusion, resulting in catastrophic global hypoxic injury, particularly in the setting of autonomic compromise.[102] Monitoring cerebral perfusion has shown some utility for the assessment of the safety of different patient positions during specific procedures, including shoulder surgery performed in the beach chair position. Cerebral perfusion as measured by both near-infrared spectroscopy and transcranial Doppler ultrasound is impaired in the seated position under anesthesia, which may contribute to an increased risk of neurologic injury.[103,104] Several studies have reported an increased incidence of marked cerebral desaturation events (regional cerebral hemoglobin oxygen saturation decrease) when shoulder surgery is performed with the patient in the seated *versus* prone position.[105–109] However, only one of these studies correlated this increased risk of cerebral desaturation with decreased performance on postoperative neurocognitive testing.[105] By contrast, prone positioning does not seem to

decrease regional cerebral hemoglobin oxygen saturation values during orthopedic surgery.[110]

Assessing Cerebral Autoregulation

Intraoperative failure of cerebral autoregulation might increase the likelihood of perioperative complications. Periods of cerebral autoregulation failure have been identified in patients undergoing noncardiac surgery. One study that included 140 patients who had major noncardiac surgery measured the optimal near-infrared spectroscopy–derived autoregulation index (tissue oxygen index) for each patient and reported that a lower optimal tissue oxygen index (*i.e.*, able to maintain autoregulation at a lower cerebral perfusion pressure) was associated with an improved rate of cognitive recovery on postoperative day 3. A higher optimal tissue oxygen index, however, was associated with reduced cognitive recovery and an increased rate of major adverse events.[111] In a study of 66 patients who had neurosurgery, tissue oxygen index values were retrospectively calculated using intraoperative blood pressure and bifrontal near-infrared spectroscopy readings.[112] Based on intraoperative tissue oxygen index values, the authors estimated the optimal perfusion pressure and compared this with the actual intraoperative arterial blood pressure. In 30 patients, the intraoperative blood pressure was found to be lower than optimal, although clinical outcomes were not evaluated.

Determination of intraoperative autoregulation has the potential to enhance perioperative management. Rather than generalizing therapeutic targets for each patient based on large-scale clinical trials, each patient can be assessed individually to determine which MAP values are likely to provide optimal perfusion of end-organs and decrease the risk of injury (that is, the optimum cerebral perfusion pressure). This approach was proven to be feasible in the setting of traumatic brain injury in the CPPopt [optimum cerebral perfusion pressure] Guided Therapy: Assessment of Target Effectiveness trial (COGiTATE), with trials in the perioperative setting underway.[113]

Postoperative Diagnostic Imaging Assessment

There is a fundamental lack of objective and reproducible testing for perioperative neurologic injury. Large clinical trials have shown that perioperative stroke and cognitive dysfunction are frequently diagnosed beyond the immediate postoperative period.[114] This finding is largely attributed to difficulties differentiating between the acute effects of surgery and anesthesia, and the complex neurologic sequelae. However, advances in neuroimaging have offered new insights into the structural basis of perioperative neurologic injury and novel avenues for early diagnosis.

Identifying Subclinical Infarction

Diffusion-weighted magnetic resonance imaging is the definitive standard for identifying acute brain infarction, with a higher sensitivity than conventional magnetic resonance imaging sequences or computed tomography.[33] Lesions are identified by the presence of cytotoxic edema, which occur within 4 h of infarction and persists for up to 2 weeks after surgery.[115] As cytotoxic edema results in intracellular swelling, the infarcted area is composed of a large volume of water, which is inherently diffusion-restricted. This direct relationship between pathology and imaging contrast is what makes diffusion-weighted magnetic resonance imaging the most sensitive and specific tool for identifying infarct in the early postoperative period.

The majority of brain infarctions are silent or covert and remain undiagnosed clinically or by neurocognitive testing, hence why the use of diffusion-weighted magnetic resonance imaging is necessary for definitive identification and quantification of these lesions. In the NeuroVISION study, 7% of 1,116 adults aged 65 yr and older and undergoing noncardiac surgery developed new ischemic lesions. These lesions were identified only by postoperative diffusion-weighted magnetic resonance imaging.[7] None of these patients were clinically diagnosed with a stroke, yet 42% experienced postoperative cognitive dysfunction in the first year after surgery. Patients with occult stroke were more likely to be diagnosed with delirium within the first 3 postoperative days and more likely to experience a subsequent stroke or transient ischemic attack than patients without new diffusion-weighted magnetic resonance imaging lesions.

Identifying Neurocognitive Disorders

Cognitive and neuropsychologic assessments are the mainstay for diagnosing postoperative cognitive dysfunction in the research setting, and additional evidence of clinical impact is essential for the diagnosis of neurocognitive disorder. However, as the definitive standard, neuropsychologic tests, are not suitable for the application in the postoperative setting, more than 60% of postoperative cognitive dysfunction and delirium cases remain undiagnosed.[19,20] Decreased performance in specific neurocognitive domains after surgery is a downstream effect of damage to the underlying neural architecture. Thus, there is growing interest in direct postoperative assessment of neurocognitive function through advanced neuroimaging.

Connectomics has clinical utility in some perioperative contexts. Notable changes in brain network topology based on diffusion tensor imaging data were identified in a population of infants undergoing surgical repair of dextrotransposition of the great vessels, and these changes were associated with subsequent reductions in academic achievement, learning, and memory during adolescence.[116] Connectomics has also been used to study cardiovascular disease more broadly in the context of metabolic syndrome and type 2 diabetes.[117,118] In patients with type 2 diabetes, functional

magnetic resonance imaging revealed decreased activity in the parietal and temporal lobes, which correlated with decreases in neurocognitive function.[119] In another study, functional magnetic resonance imaging changes were noted before structural changes, indicating that developing characteristic functional magnetic resonance imaging signatures might enable improved and earlier diagnosis of cognitive dysfunction.[118] However, replicable brain signatures of postoperative cognitive dysfunction and their association with (and the ultimate impact of) magnetic resonance imaging–defined brain infarction have not yet been established.

A 2019 study identified white matter structural features that underly cognitive and emotional function in healthy adults using diffusion magnetic resonance imaging (fig. 7).[77] An assessment of cerebral tissue with diffusion kurtosis imaging revealed that emotional dysregulation was associated with increased diffusivity and reduced cellular complexity in specific regions of the temporal and parietal lobes, as well as the adjoining white matter tracts. Emotional dysregulation is a factor contributing to morbidity in postoperative cognitive dysfunction,[120] and using diffusion kurtosis imaging in the context of perioperative medicine will likely provide novel insights into the structural basis of this disease. For example, diffusion kurtosis imaging has previously revealed sensitive and specific white matter changes underlying depression, concussion, and motor neuron disease.[121–123] Such understanding, and the development of replicable, robust, and objective markers of postoperative cognitive dysfunction, will help identify at-risk individuals, facilitate the development of preventive therapies, enable early intervention, and overcome the insensitivity and subjectivity of the current definitive standard psychometric testing.

Conclusions

Neurologic injury after noncardiac surgery is associated with marked morbidity and mortality and is responsible for an immense global healthcare burden. Research and expert consensus statements continue to highlight the desire for improved hemodynamic optimization during surgery, but the role of this strategy to improve outcome is uncertain. Novel neuroimaging modalities have the potential to revolutionize perioperative care by providing real-time, noninvasive measurements of end-organ perfusion with potential for early detection of insult and prevention of perioperative injury. However, advanced neuroimaging methods have predominantly been developed and evaluated in critical care settings, particularly in the context of neurotrauma, and there is a paucity of studies that assess application of these methods in the perioperative setting. Further research using novel neuroimaging techniques and derived biomarkers in perioperative practice is essential to outline both the pathophysiology of organ injury and the utility of brain monitoring–directed therapy.

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Competing Interests

The authors declare no competing interests.

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MIND TO MIND

Creative writing that explores the abstract side of our profession and our lives

Stephen T. Harvey, M.D., Editor

Swan Song

Lealani Mae Y. Acosta, M.D.

She warbled, "In that sweet by and by…" trailing
off, dissolving into gasps for air,
still smiling, hand perched on the bedrail, frail.
Each breath was precious, not a word to spare.
"I never had much voice to speak of, even
when I could catch my breath," she fluttered.
The IV morphine gave her some reprieve.
"Oh, please, won't you hold my hand?"
Smiles, uttered.
"I'm actually just a student doctor, ma'am."
I felt a witless, useless dodo.
Books
could not have prepped me for this last exam.
I didn't want to fail her. She then took
my hand in hers. "No need to stay too long."
My silence harmonized with her swan song.

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MIND TO MIND

Creative writing that explores the abstract side of our profession and our lives

Stephen T. Harvey, M.D., Editor

The Burnout Dance of the Forever Pandemic

Vidya T. Raman, M.D., M.B.A., Vanessa A. Olbrecht, M.D., M.B.A.,
Allison M. Fernandez, M.D., M.B.A.

Day in, day out, the mask goes over my face, nose, and mouth

Not since I was a teen have I had to use acne wash

Pain everywhere, especially behind my ears and the bridge of my nose

My roots are gray and the ends are scraggly

Who will see, who will care?

Time is precious in a way never before but meaningless

The list is never ending of the things to get done

One item comes off and another immediately added on

From the badge swipe into the operating room till the badge swipe out in the parking garage,

I count and measure the time with each minute

But the day is a blur

I cannot tell you if it was a good day or a bad day

Just a day, another day

A lonely life surrounded by many but alone with my swirling thoughts and worries

One hundred texts from home—we are out of milk, we need bread, when are you coming

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Thank goodness for delivery service, one flick of my finger on my phone and done

I wish everything was that easy

There is the faculty Zoom meeting—I plan to multi-task while watching soccer practice

Will they remember if I was present at either place?

Do what you can when you can

When does this happen?

Survey after survey

Are you well?

No and no, and not at all

But what is the solution besides being quiet

Who has time to contest when each day is a battle

To be fought not to be lost but never won

Of work, home, wash, rinse, repeat

When was science so reviled?

When did fact become lie and the hero now the villain?

A bad doctor, mother, wife, daughter, sister

Not one role a success

I was forced to attend this macabre dance

Had no choice or will

Which seems to go on and on

Please give me the strength to survive one more dance

Of the forever pandemic

Evolution of Anesthesia Patient Safety Movement: Comment

To the Editor:

I read with great interest the excellent article on the Patient Safety Foundation.[1] However, I think that your readers may be interested to learn of the origin of efforts to improve quality of care and patient safety.

It began in the 1960s when the Board of Governors of the American College of Anesthesiologists, under the leadership of Dr. Tom Burnap, assumed the responsibility for evaluating quality of care and patient safety in anesthesiology. Members of the American College of Anesthesiologists attended national conferences on quality assessment to learn and apply the methodology to anesthesiology. These early activities led President "Rick" Siker to appoint a new committee on quality of care. I served as chair of the quality of care committee for 2 yr.

The committee developed criteria for evaluating quality of care, engaged in on-site inspection of departments of anesthesia at the request of hospital administrators, and advanced the concept of "practice parameters." When I became president during 1980 and 1981, the title of my presidential address was "Quality of Care: ASA's *Raison d'Etre*." Anesthesiology was the first medical specialty to develop a formal program for evaluating quality of care.

A few years later, "Jeep" Pierce established the Patient Safety Foundation, which elevated quality of care and patient safety to a whole new level.

Competing Interests

The author declares no competing interests.

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Evolution of Anesthesia Patient Safety Movement: Reply

In Reply:

We thank Dr. Brown for his insights regarding the integration of nascent quality of care methodologies and initiatives into the American College of Anesthesiologists in the 1960s (both organizations were located in Park Ridge, Illinois, in the 1960s and currently are located in Schaumburg, Illinois).[1] We had not included that information in our review of the evolution of the anesthesia patient safety movement.[2] As he appropriately notes, the development of quality of care activities provided a crucial contribution to the onset of the anesthesia patient safety movement in the 1980s. Drs. Brown, Siker, and others played important leadership roles in both organizations and advocated for the quality of care activities that would subsequently serve as the foundation of the movement. They appropriately should be credited for their diligent efforts to improve quality of care in anesthesiology.

The link between anesthesiology and patient safety arguably goes back to the death of 15-year-old Hannah Greer on January 28, 1848, during a chloroform anesthetic.[3] One hundred years ago, in its inaugural issue, *Current Researches in Anesthesia & Analgesia* (subsequently named *Anesthesia & Analgesia*) published an anesthesia patient safety article, "Morbidity and Mortality in Obstetrics as Influenced by Anesthesia."[4] Numerous studies on anesthesia-related mortality and morbidity followed. In 1978 through the 1980s, Jeffrey B. Cooper, Ph.D., John H. Eichhorn, M.D., and colleagues introduced the concepts of standards of patient monitoring and the study of human factors and critical incidents in analyses of anesthesia errors and mishaps.[5–8] These concepts provided the specialty with new opportunities to improve patient safety.

This progression of new knowledge and approaches to patient safety, coupled with the preexisting organizational advocacy for quality of care described, in part, by Dr. Brown, provided the basis for the specialty to be able to respond to the swell in public interest in anesthesia patient safety that arose from the 1982 ABC television network's *20/20* production "The Deep Sleep: 6,000 Will Die or Suffer Brain Damage"[9] and to a concomitant growing medical malpractice insurance crisis for anesthesiologists in the United States. It was these unique challenges, in our opinion, that led to a sharp demarcation in 1982 between the previous steady but slowly progressive efforts to improve quality of care and the new tsunami of interest in rapidly developing and implementing a distinct anesthesia patient safety movement. Therefore, it is this period starting in 1982 that we designated for the purposes of our article as the start of the anesthesia patient safety movement.

Competing Interests

The authors declare no competing interests.

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Pressure Support Ventilation and Atelectasis: Comment

To the Editor:

We read with great interest the article by Jeong *et al.*[1] titled "Pressure Support *versus* Spontaneous Ventilation during Anesthetic Emergence—Effect on Postoperative Atelectasis: A Randomized Controlled Trial." Although many studies have looked at the potential effects of various intraoperative open lung ventilation strategies on postoperative pulmonary outcomes, recent evidence suggests that their potential benefits may be limited if no action is taken to minimize lung derecruitment during the emergence period.[2] Considering that postoperative atelectasis plays a central role in the development of postoperative pulmonary complications, and that maintaining positive pressure during emergence may help preserve lung aeration,[3] the research question of Jeong *et al.* is of paramount importance. However, we have some concerns regarding key aspects of the study's methodology.

First, we were especially worried about elements used to define and measure the incidence of atelectasis, the study's primary outcome. The authors' definition (more than three lung sections with a non-zero atelectasis score) is not standard[4] and has not been previously validated. Can the authors specify whether their definition was selected before conducting the study to reassure readers on the absence of data-driven threshold selection? Performing sensitivity analyses looking at different thresholds for the number of atelectatic lung sections necessary to classify the outcome would better assess the robustness of their findings.

Second, we were puzzled to read that Jeong *et al.* not only used a modified and unvalidated echographic pulmonary aeration loss score[5] but also introduced their own modifications, potentially further weakening the validity of their primary outcome classification. In particular, loss of lung sliding with lung pulse is not a sign of atelectasis but rather a sign of a well-aerated lung without ventilation. This finding could have indicated the presence of a mucous plug which may have been resolved after a simple coughing fit without causing any atelectasis. Including this sign in their atelectasis score seems problematic. We encourage the authors to use the lung ultrasound score, a validated echographic loss of aeration score, to report their results.[6]

Third, their study was underpowered for their anticipated effect size. Using the same assumptions (an incidence of 53% in the control group and 37% in the intervention group for an absolute estimated effect of 16%), we calculated that a sample size of 302 patients would have been necessary even before considering a 15% dropout rate. Their greater-than-anticipated observed effect explains why their results achieved statistical significance. However, underpowered studies are prone to inflated results with positive results that are more likely to be false positives.[7]

Fourth, the authors' definition of hypoxemia, a secondary outcome, may lead to missing important clinical effects resulting from their intervention. A punctual event of oxygen saturation measured by pulse oximetry greater than 92% may be not be clinically significant in comparison with a prolonged postoperative need for high fractional inspired oxygen tension. Can the authors provide data on this secondary outcome using a time-weighted need for organ support, such as oxygen-free days or cumulative postoperative oxygen administration?

The imaging study by Jeong *et al.* is an essential first step in clarifying the role of assisted ventilatory modes during anesthesia emergence. However, there is still a lot of work to be done to answer the salient question: Are assisted ventilatory modes an important part of an open lung strategy at emergence that may lead to a decreased incidence of postoperative pulmonary complications?

Competing Interests

Dr. Girard is a paid consultant for the point-of-care ultrasonography group of GE Healthcare (Milwaukee, Wisconsin). The other authors declare no competing interests.

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Pressure Support Ventilation and Atelectasis: Reply

In Reply:

We very much thank Zaouter *et al.* for their interest[1] in our research, "Pressure Support *versus* Spontaneous Ventilation during Anesthetic Emergence—Effect on Postoperative Atelectasis: A Randomized Controlled Trial."[2]

The main questions of Zaouter *et al.* were why we defined atelectasis only when there were signs of atelectasis in three or more lung sections and why we used a modified scoring system to evaluate atelectasis severity. For the first question, as the authors noted, there is no established definition of atelectasis diagnosed by ultrasonography. We thought that at least 25% (3 of 12 sections) of lung areas should show signs of atelectasis to be a clinically significant atelectasis because almost all patients showed an atelectasis sign in at least one lung section. We admit that 25% of lung sections is arbitrary, but this threshold was determined before conducting the study.

For their second question, we thought that an atelectasis scoring system focused on anesthesia-induced atelectasis was needed because many protocols were developed for

intensive care unit patients in previous studies.[3–6] However, our protocol is still based on the protocols that are widely used.[6,7] Anesthesia-induced atelectasis did not show definite B lines, which were used in the previous scoring system (B lines: hyperechoic vertical lines starting from the pleural line with the length of 8 cm or longer). Rather, anesthesia-induced atelectasis showed subpleural consolidations with short vertical lines starting from the margin of consolidation (pseudo B lines).[7] Accordingly, loss of A line with multiple subpleural consolidations has been reported as a more common and helpful finding to diagnose anesthesia-induced atelectasis.[7] In consideration of the development process of anesthesia-induced atelectasis, the grade 3 atelectasis, which is "loss of lung sliding and appearance of lung pulse," was added to our grading system. We found that the collapse of small bronchioles and alveoli leads to "loss of lung sliding and appearance of lung pulse" as subpleural consolidation progresses to a larger parenchymal consolidation.[8] This was also reported in previous studies.[8] Although we modified the scoring system for a more accurate diagnosis of anesthesia-induced atelectasis, it was not validated. We described this in the limitations to our study.

For the third question (sample size), we found that the power of our study did not meet the expectations and needed a larger number of patients. However, we understand that the probability of type II error (false negative) would have decreased as the sample size (power) increased, but the type I error (false positive) usually remains the same.[9] Therefore, we think our positive results would have been confirmed with more power if the sample size had increased.

We agree with Zaouter *et al.* that oxygen-free days or cumulative postoperative oxygen administration may be more important than the incidence of hypoxia as a secondary outcome. However, most patients received oxygen administration only on the night of surgery, and there was no difference in postoperative complications such as pneumonia and hospital stay between the two groups. So, we cautiously speculate that the time-weighted need for oxygen support would not have been different between the two groups.

Competing Interests

The authors declare no competing interests.

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Targeting Depth of Anesthesia to Prevent Delirium: Comment

To the Editor:

Brown *et al.*[1] nicely described their work comparing spinal anesthesia with targeted sedation based on Bispectral Index values compared with general anesthesia

(masked Bispectral Index) and the outcome of delirium. Intraoperative hypotension has been associated with delirium.[2,3] The adjusted hazard ratio associated with a 1-mmHg increase in time-weighted average of mean arterial pressure less than 65 mmHg was 1.11 (95% CI, 1.03 to 1.20).[2] The study by Brown *et al.*[1] found that the lowest mean arterial pressure was similar in both groups (general anesthesia, 59 [51 to 64] *vs.* spinal anesthesia, 60 [52 to 64]); however, the relationship between intraoperative hypotension and the subsequent development of delirium might be more of a cumulative exposure response than a single threshold. It would be of interest to know the hypotension exposure by time under the 65- or 60-mmHg threshold and to consider that in a treatment-by-covariate interactions analysis. In short, we appreciate Brown *et al.* for their great contributions to this important topic.

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Competing Interests

The authors declare no competing interests.

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Targeting Depth of Anesthesia to Prevent Delirium: Comment

To the Editor:

We read with great interest the recent article by Brown *et al.*[1] regarding the impact of Bispectral Index (BIS)–guided sedation on the incidence of postoperative delirium during spinal anesthesia for spine surgery compared with BIS-masked general anesthesia. We appreciated the originality and efforts of the authors to clarify a still controversial topic such as the connection between depth of the hypnotic component of anesthesia and postoperative delirium. Nevertheless, after careful reading of the trial and its conclusions, we would like to address some critical input to the authors.

First, in the "Materials and Methods" section, the authors report that they achieved spinal anesthesia using intrathecal bupivacaine or lidocaine. Spinal anesthesia with lidocaine carries the risk of transient neurologic symptoms.[2] Transient neurologic symptoms constitute an acute pain syndrome that could exacerbate postoperative pain and thus increase the incidence of postoperative delirium. In addition, acute postoperative urinary retention after spinal anesthesia could increase pain and discomfort and contribute to postoperative delirium.[3] Both complications could have influenced the reported results on postoperative delirium, but their incidence is not reported by the authors.

Second, we would have been happy to see more information on intraoperative hemodynamic stability, all the more since moderately high doses of intrathecal local anesthetic agents and prone position for surgery may have an impact on it. Despite the fact that the relationship between intraoperative hypotension and the incidence of postoperative delirium is still not clearly established, it would have been informative to report not only on the lower intraoperative mean arterial pressure but also, and more importantly, on the decrease from initial mean arterial pressure and time spent below a patient-adapted threshold value, *i.e.*, the time of relative cerebral hypoperfusion.[4,5] Indeed, cerebral perfusion

pressure beyond the autoregulatory limit is an independent risk factor for the development of neurologic complications, including postoperative delirium. Rightly, the authors report the incidence of postoperative stroke in their trial, but several publications have emphasized the importance of subclinical cerebral vascular events and their potential role in generating postoperative delirium.[6]

Third, and in accordance with the results of this study, the BIS is probably not the right tool to guide anesthesia depth with the aim of avoiding postoperative delirium. Drug-induced alterations of brain function are complex, and BIS catches only a very small part of them.[7] However, this does not mean that we should not seek a better understanding of the changes that are really relevant with regard to postoperative delirium and that should be prevented. The electroencephalogram is certainly the most accessible and noninvasive tool to be used in this respect, and several teams are currently performing an in-depth analysis of intraoperative electroencephalogram data to find out the most relevant markers. This must occur in a more general framework that takes account of the multifactorial nature of postoperative delirium, in which factors such as neuroinflammation, quality of organ perfusion, drug interactions, adequacy of antinociception, and patient comfort intervene.

Therefore, we should consider BIS as reflecting the tip of the iceberg only, while the immense mountain of ice remains hidden. Using BIS as the sole tool to prevent postoperative delirium is piloting a boat like the *Titanic*'s captain: the most important part of the problem could still be under the surface of the water.

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Competing Interests

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Targeting Depth of Anesthesia to Prevent Delirium: Reply

In Reply:

We thank Drs. Xiong *et al.*[1] and Carella and Bonhomme[2] for their comments regarding the shaping anesthetic techniques to reduce post-operative delirium (SHARP) study.[3] We agree that evaluating intraoperative hypotension as a potentially moderating factor in the SHARP trial would be interesting. We found no difference in the number of minutes at a mean arterial pressure less than 55 mmHg between the spinal anesthesia with targeted sedation group (median, 0 min; interquartile range, 0 to 5) and the general anesthesia group

(median, 0 min; interquartile range, 0 to 5; $P = 0.51$). Further, when the number of minutes at a mean arterial pressure less than 55 mmHg (considered as a categorical variable) was added to the main regression model as an interaction term, the interaction term was not significant, indicating that mean arterial pressure less than 55 mmHg did not modify the effect of anesthetic choice on postoperative delirium. Finally, the number of minutes at a mean arterial pressure less than 55 mmHg was not associated with delirium when added to the adjusted model described in the article.[3] We did not prospectively record hypotension exposure below a 60- or 65-mmHg threshold but will consider this for potential future studies.

A further question was whether transient neurologic symptoms or urinary retention after spinal anesthesia could have caused increased pain or discomfort and thus influenced the development of postoperative delirium. Since the pain scores were similar on postoperative day 1 between groups, we do not think these complications were strong factors that could have biased the study results. Finally, we agree that Bispectral Index may not be the optimal tool to guide anesthesia depth, and further work using the electroencephalogram is needed. However, we designed this study based on previous studies that suggested a beneficial role for Bispectral Index in guiding anesthetic depth, and we utilized an anesthetic regimen that allowed for sedation to a level lighter than general anesthesia, irrespective of BIS levels.

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Competing Interests

Dr. Brown has a data share agreement with Medtronic (Minneapolis, Minnesota) in unrelated areas. Dr. Hogue consulted for and received grant support from Medtronic in unrelated areas.

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Cohen's Comprehensive Thoracic Anesthesia

Edited by Edmond Cohen, M.D., F.A.S.A. Philadelphia, Elsevier, Inc., 2022. Pages: 825. ISBN: 978-0-323-72092-2. Price: $201.50 (Hardcover).

Edmund Cohen, M.D., F.A.S.A., longtime professor of anesthesiology at the Icahn School of Medicine at Mount Sinai (New York, New York), has made a monumental contribution to the field of thoracic anesthesiology with the publication of the new textbook *Cohen's Comprehensive Thoracic Anesthesia*. He has assembled an international team of experts who take the reader on a journey through the history of operating in the thorax from the days of the first-century Roman author Celsus to the latest in minimally invasive techniques, enhanced recovery principles, protective ventilation strategies, lung transplantation, and lung isolation tools of all kinds.

The book's utility is enhanced by its link to an electronic version, which enables the reader to access it on any electronic device, search topics, enlarge images, highlight content, and take notes. This electronic feature is equally invaluable for the resident or fellow who needs immediate clinical answers, for the academic faculty member who has just been asked a question that he or she cannot readily answer, or for the occasional thoracic anesthesiologist in the community setting who seeks a quick refresher in troubleshooting a double-lumen endotracheal tube or guidance in how to handle an urgent thoracic problem.

As might be expected from Dr. Cohen, who is internationally recognized for his expertise in lung isolation and the development of the Cohen endobronchial blocker (Cook Medical, USA), there is ample information about selection of lung isolation devices, including double-lumen tubes and endobronchial blockers. Co-author Javier Campos, M.D., provides a thorough and unbiased review of the advantages and disadvantages of commercially available devices, with excellent illustrations including diagrams, photographs, radiographic and ultrasound imaging, and bronchoscopic views. We concur with the assessment of indications and guide to positioning for right-sided double-lumen tubes. A separate and very helpful chapter, also by Dr. Campos, is devoted to the practical real-world scenarios of the patient with a difficult airway for intubation, the need for tube exchange from single- to double-lumen tube and vice versa, and the problem of lung isolation in the patient with a tracheostomy tube.

The chapter on mediastinal mass and superior vena cava syndrome is well referenced and provides a case presentation that illustrates many of the problems with this frightening clinical challenge. In our opinion, the goal in these scenarios more often is to avoid giving general anesthesia (at least for the adult patient) than to do it and to obtain a tissue diagnosis with as minimal intervention as possible. Many of the most severe cases result from lymphoma and are treated with chemotherapy and/or radiation therapy rather than by surgical resection once a tissue diagnosis has been established. The anesthesiologist's role may be primarily to counsel against the choice of a general anesthetic in a severely compromised patient as the referring physicians may be unaware of the risks. Lesions such as thymomas typically are smaller and less likely to cause respiratory or circulatory compromise, so the anesthetic challenges are correspondingly less. However, the two authors perform a valuable service in outlining every step that should be considered and planned for, including rapid implementation of cardiopulmonary bypass, if general anesthesia cannot be avoided in the patient who is symptomatic from a large mediastinal mass.

True to its title, the textbook focuses on noncardiac anesthesiology, but it acknowledges the overlap into the cardiac subspecialty with chapters on extracorporeal ventilatory therapies, thoracic aneurysm repair, the management of lung transplantation, and the application of transesophageal echocardiography in the thoracic setting. The chapter on lung resection in patients with pulmonary hypertension succinctly reviews the management of right ventricular failure, although more attention might have been given to discussing the utility of transesophageal echocardiography as a monitor and inhaled pulmonary vasodilators as therapy. The discussion of thoracic aneurysms is confined to the ascending aorta, with no mention of cannulation and partial bypass techniques for repair of the descending aorta. Cerebral protection is discussed, but spinal cord protection and neurologic monitoring are not.

There are instances in this overall outstanding textbook where the reader would wish for fewer platitudes and more precision, as in this sentence, which could be applicable to any case: "Therefore, perioperative hypotension, tachyarrhythmias, or anemia should be avoided to reduce the incidence of serious adverse cardiovascular events." The use of thoracic epidural analgesia is endorsed more than once as the "gold standard" for analgesia with scant mention of the sympathetic blockade and hypotension that may lead our surgical colleagues to object to it. In the chapter on the postoperative management of acute pain, there is no mention of the use of intrathecal narcotics, which may be a valid option in selected circumstances for analgesia that can reduce total

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systemic opioid consumption with less relative risk of hypotension. (Intrathecal opioids are listed as an option in the chapter on thoracic trauma management.) The chapter on tracheal resection and reconstruction describes the use of a superglottic airway in an excellent discussion on the need for smooth emergence and extubation but fails to mention the laryngeal mask airway as a useful option during the initial stage of resecting a high tracheal or cricothyroid lesion before cross-table ventilation has been instituted.

These minor criticisms aside, Dr. Cohen and his colleagues have contributed enormously to the armamentarium of teaching thoracic anesthesiology. This book lives up to its claim of being comprehensive and detailing the highest standards of thoracic anesthesia care as they exist in 2022. This is indeed a book that no academic center should be without and that everyone who administers thoracic anesthesia may wish to add to their personal libraries.

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