ANESTHESIOLOGY

Reduced Sensitivity to Anesthetic Agents upon Lesioning the Mesopontine Tegmental Anesthesia Area in Rats Depends on Anesthetic Type

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Lesions of the mesopontine tegmental anesthesia area in the brainstem render rats strongly insensitive to pentobarbital
- The effects of mesopontine tegmental anesthesia area lesions on responses to other anesthetics have not been previously reported

What This Article Tells Us That Is New

- Targeted microinjection of ibotenic acid into the mesopontine tegmental anesthesia area in adult rats led to an up to twofold loss in anesthetic potency of etomidate and propofol
- In contrast, the potency of ketamine, medetomidine, and alfaxolone/alfadolone was unaffected
- These observations suggest that the mesopontine tegmental anesthesia area of the brainstem may serve as a key structure to selectively mediate transition from wakefulness into an anesthetic state in response to γ-aminobutyric acid–mediated anesthetics

ABSTRACT

Background: The brainstem mesopontine tegmental anesthesia area is a key node in circuitry responsible for anesthetic induction and maintenance. Microinjecting the γ-aminobutyric acid–mediated (GABAergic) anesthetic pentobarbital in this nucleus rapidly and reversibly induces general anesthesia, whereas lesioning it renders the animal relatively insensitive to pentobarbital administered systemically. This study investigated whether effects of lesioning the mesopontine tegmental anesthesia area generalize to other anesthetic agents.

Methods: Cell-selective lesions were made using ibotenic acid, and rats were later tested for changes in the dose–response relation to etomidate, propofol, alfaxalone/alfadolone, ketamine, and medetomidine delivered intravenously using a programmable infusion pump. Anesthetic induction for each agent was tracked using five behavioral endpoints: loss of righting reflex, criterion for anesthesia (score of 11 or higher), criterion for surgical anesthesia (score of 14 or higher), antinociception (loss of pinch response), and deep surgical anesthesia (score of 16).

Results: As reported previously for pentobarbital, on-target mesopontine tegmental anesthesia area lesions reduced sensitivity to the GABAergic anesthetics etomidate and propofol. The dose to achieve a score of 16 increased to 147 ± 50% of baseline in control animals ± SD ($P = 0.0007$; 7 lesioned rats and 18 controls) and 136 ± 58% of baseline ($P = 0.010$; 6 lesioned rats and 21 controls), respectively. In contrast, responsiveness to the neurosteroids alfaxalone and alfadolone remained unchanged compared with baseline (94 ± 24%; $P = 0.519$; 6 lesioned rats and 18 controls) and with ketamine increased slightly (90 ± 11%; $P = 0.039$; 6 lesioned rats and 19 controls). The non-GABAergic anesthetic medetomidine did not induce criterion anesthesia even at the maximal dose tested. The dose to reach the maximal anesthesia score actually obtained was unaffected by the lesion (112 ± 8%; $P = 0.063$; 5 lesioned rats and 18 controls).

Conclusions: Inability to induce anesthesia in lesioned animals using normally effective doses of etomidate, propofol, and pentobarbital suggests that the mesopontine tegmental anesthesia area is the effective target of these, but not necessarily all, GABAergic anesthetics upon systemic administration. Cortical and spinal functions are likely suppressed by recruitment of dedicated ascending and descending pathways rather than by direct, distributed drug action.

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General anesthetic agents reliably induce transitioning from wakefulness to unconsciousness, permitting pain-free surgery. The transition is dramatic and stereotyped, involving loss of awareness, loss of memory formation, immobility, and loss of sensory perception. Given the widespread and homogeneous dispersion of the agents by the circulatory system and the ubiquity of their cognate receptors, global functional suppression is to be expected. However, suppression is not uniform. Some brain functions are only moderately altered during general anesthesia including respiration, autonomic regulation, and primary cortical processing. "Line detectors" in visual cortex and somatosensory columns, for example, were discovered in

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anesthetized animals. This implies heterogeneity in the central nervous system (CNS) site(s) suppressed during anesthesia notwithstanding the ubiquitous distribution of the molecules themselves.

Indeed, it is widely held among investigators[1–5] that the different functional constituents of the anesthetic state are seated at widely separated brain locations. Thus, anesthetic molecules that bind to receptors in the cerebral cortex are presumed to be responsible for loss of consciousness and amnesia, whereas receptor binding in the spinal cord is responsible for motor and somatosensory suppression. Respiratory and autonomic regulation, in contrast, is mostly seated in the medulla, perhaps at locations that are relatively insensitive to anesthetic molecules.

Competing with this global or patch-wise suppression hypothesis is a very different model, the dedicated pathways hypothesis.[6–8] While acknowledging the widespread distribution of anesthetic agents and their receptors, this hypothesis posits that anesthetic induction is due to primary drug action within the circuitry that realizes cycling between wakefulness and sleep. Anesthetics substitute for an endogenous neurotransmitter. The actual transitions in brain state are brought about secondarily by recruitment of dedicated ascending and descending axonal pathways. Thus, cortical and medullo-spinal functions are selectively modulated by synaptic action rather than by pharmacodynamic action of the anesthetic molecules themselves. It was French *et al.*[9] and Magni *et al.*[10] who first proposed that anesthetics might co-opt sleep/arousal circuitry in the brainstem reticular activating system. Contemporary studies have tried to define more precisely where and how this occurs.[6,7,11–15]

Using a systematic microinjection approach, we advanced the dedicated pathways idea with the discovery of a small nucleus, the mesopontine tegmental anesthesia area, which appears to be a key node in circuitry that enables anesthetic induction.[16–18] Briefly, we showed the following: (1) Delivering the γ-aminobutyric acid–mediated (GABAergic) anesthetics pentobarbital and propofol into the mesopontine tegmental anesthesia area, or the specific $GABA_A$-receptor agonist muscimol, quickly and reversibly induces an anesthesia-like state closely resembling systemically induced general anesthesia (http://links.lww.com/AA/B466). The minute doses used and the rapid onset time preclude the possibility of agent redistribution to the spinal cord and cortex.[18] (2) Lesions of this area reduce the sensitivity of rats to systemic pentobarbital such that anesthesia can no longer be induced at clinically relevant doses.[19] Because the rest of the CNS continues to be exposed to normal anesthetic concentrations of the pentobarbital, but anesthesia is not induced, this observation challenges the hypothesis of global or patch-wise suppression. The primary aim of the present study was to evaluate the effect of mesopontine tegmental anesthesia area lesions on anesthetic induction using agents other than pentobarbital.

Materials and Methods

Animals and Surgery

Animals. We used adult (more than 250 g, 2.5 to 3.0 months of age) Wistar-derived Sabra strain rats, all males except for eight females used to probe the possibility of a striking sex difference. The rats were maintained under specific pathogen-free conditions at 21 to 22°C, 12 h:12 h day:night cycle (lights on at 7:00 AM), with water and food pellets available *ad libitum*. The experiments were approved by the Institutional Animal Care and Use Committee of the Institute for Life Sciences of the Hebrew University of Jerusalem (Jerusalem, Israel) and were conducted in accordance with guidelines of the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals.

Surgery. Two surgical procedures were employed: (1) placement of an iv catheter in the femoral vein and (2) an intracranial procedure targeting the mesopontine tegmentum. The operations were separated by ~2 weeks (13.7 ± 9.2 days). In the first procedure, carried out under chloral hydrate anesthesia (400 mg/kg intraperitoneal; Sigma–Aldrich, Israel), the animals were implanted with a femoral vein catheter that was exteriorized behind the neck and sealed with a sterilized stainless steel plug (additional details in Minert and Devor[19]). The catheter was filled with heparin in sterile saline (100 units/ml, Kamada Ltd., Israel). After catheterization, the rats were transferred to individual cages. The heparin solution was flushed and refilled every 2 to 3 days.

Intracranial procedures were carried out under ketamine and xylazine anesthesia (10.2 mg/kg intraperitoneal ketamine (Ketaset, Fort Dodge Animal Health, USA) and 3.6 mg/kg intraperitoneal xylazine (Sedaxylan, Eurovet, The Netherlands) supplemented with propofol (~10 mg/kg iv; 1% Diprivan, Astra Zeneca, USA) to maintain immobility if required. After craniectomy, six 50-nl volumes of 1% ibotenic acid (Tocris, USA; in normal saline containing 1% pontamine sky-blue dye as a marker) or vehicle (saline and dye) were microinjected stereotaxically through a fine glass micropipette in experimental and control rats, respectively (see Minert and Devor[19] for details). Three microinjections targeting the mesopontine tegmental anesthesia area were made on each side, aimed at left–right symmetrical skull-level coordinates: 7.6, 8.2, and 8.8 mm caudal to bregma, ± 1.25 mm lateral to the midline, and 6.3 mm below the dura. In addition, five rats received lesions intentionally placed off-target, in the dorsal thalamus (3.4, 4.0, and 4.6 mm caudal to bregma; ± 3.0 mm lateral to the midline and 4.0 mm below the dura). Ibotenic acid is an excitotoxin that kills neurons that bear glutamate receptors while sparing passing axons that do not.[20]

Experimental Groups. All animals, except as noted, were catheterized, and some of them went on to have an intracranial procedure. The latter were assigned to experimental groups based on whether or not a lesion was made, and the extent

to which it encroached on the mesopontine target region in a symmetrical manner. That is, assignment was not based on intent at the time of surgery, but rather on the lesion outcome established retrospectively by postmortem histologic analysis by an observer blinded to the experimental results. The evaluation procedure is described below (Histology section).

The "lesion group" consisted of nine animals in which the left–right symmetrical portion of the lesion covered more than 35% of the counting frames of the mesopontine tegmental anesthesia area (range, 37.5 to 79.5%). "Control group" animals comprised two subgroups: 43 rats with venous catheter but no craniectomy or intracranial procedure (the "catheter-only" group) and another 13 in which vehicle was microinjected into the mesopontine tegmental anesthesia area ("vehicle group") for a total of 56 control group rats. Use of more control animals than experimentals is a strategy for economizing on difficult-to-obtain experimental subjects.[21] Finally, the "off-target group" included 15 rats in which an ibotenic acid lesion was carried out. In five of these animals, the lesion was intentionally placed off-target, as noted above under "Surgery." In the remainders the lesion missed the mesopontine tegmental anesthesia area or encroached on it only slightly. All animals in all three groups underwent drug testing, although not all were tested with all five study agents (table 1). Details of group allocation and the testing pipeline followed for each rat are given in table 2. A number of additional animals as noted below were used to "service" the main study. They were not included among the three experimental groups.

Testing Routine

Anesthetics. The anesthetics tested were etomidate, propofol, alfaxalone/alfadolone, ketamine, and medetomidine (table 1). Comparison data for pentobarbital using the same methods are available in a previous publication[19] in which we evaluated 17 lesioned animals including the nine lesion group rats used here. Some lesion group, control group, and off-target group animals were tested both before and after lesioning. For others, testing began only after the intracranial procedure was carried out (table 2).

Beginning 7 days or more after catheterization and/or the completion of the intracranial procedure, the rats were placed in a 40 × 25–cm enclosure (height, 20 cm), and the catheter end was connected to a syringe *via* a length of polyethylene tubing (PE50). First, the catheter dead space (50 μl) was filled with the test drug, flushing the saline–heparin into the circulation. Then we began administering the test anesthetic using a digital infusion pump (KD Scientific, USA) with a delivery rate that had previously been found in the service rats to induce deep surgical anesthesia in 15 to 20 min. The delivery rate was individually adjusted to the animal's body weight. Anesthetic was delivered for 60 s and then paused for 60 s while the depth of anesthesia was scored. A second 60-s infusion followed, then a second scoring period, and so forth until the anesthesia score reach the maximum of 16. Infusion was then stopped, and the catheter was flushed and resealed. After recovery of the righting reflex, the animals were returned to their home cages. Throughout the testing period, the body temperature of the animals was maintained with a radiant heat lamp.

Dose–Response Relation and Reproducibility. Anesthesia was scored on a 0 to 16-point behavioral scale (Supplemental Digital Content 1, http://links.lww.com/ALN/C142).[16,19] Briefly, the score summed two motor scales that graded posture and righting reflex and two sensory scales that graded response to calibrated tail and foot pinch (nociception). Each scale ranged from 0 (normal response during wakefulness) to 4 (no response). Anesthetic endpoints used are noted below. Testing was done at a fixed time of day (10:00 AM to 2:00 PM) by a single observer who was aware of the drug administered and whether a craniectomy had been carried out but not the extent or location of the lesion, if any. To estimate reliability across observers, in three rats using three different drugs, scoring was done independently by a second observer, yielding nearly identical results.

In addition to pentobarbital, each of the 9 lesion group rats was tested on most of the 5 additional agents (mean, 4.3; table 2). Each agent, in turn, was evaluated in at least 5 of these rats (mean, 6.0 ± 0.7). Individual control group rats were tested with 1 to 4 of the 5 agents (mean, 1.7 ± 0.9; 18 to 21 rats/agent), and rats with off-target lesions were tested on 1 to 4 agents (mean, 2.3 ± 1.3; at least 5 rats/agent). On a random basis, 18 of the 43 animals tested after catheterization went on to have one of the two intracranial procedures. Additional animals were catheterized and

Table 1. Anesthetic Agents Used and Rates of Intravenous Delivery

Anesthetic Agent	Manufacturer or Supplier	Concentration, mg/ml	Delivery Rate, $mg \cdot kg^{-1} \cdot min^{-1}$
Etomidate-Lipuro	B. Braun Melsungen, Germany	2	0.5
Propofol (1%, Diprivan)	Astra Zeneca, USA	10	2
Alfaxalone/alfadolone (Saffan)	Glaxovet, Canada	9/3	1.8/0.6
Ketamine (Ketaset)	Fort Dodge Animal Health, USA	100	10
Medetomidine hydrochloride (racemic, Domitor)	Pfizer, USA	0.1	0.008

Table 2. Composition of the Experimental Groups

			Before Craniectomy						After Craniectomy					
		Rat No.	Pentobarbital	Etomidate	Propofol	Alfaxalone/ Alfadolone	Medetomidine	Ketamine	Pentobabital	Etomidate	Propofol	Alfaxalone/ Alfadolone	Medetomidine	Ketamine
Control group	Catheter-only subgroup	199	1					2						
		200	1					2						
		205	1					2						
		215†			1,2,3									
		217	2					1						
		222†			4			1,2,3						
		223†						1,2,3						
		224†						1,2,3						
		225†			4			1,2,3						
		226†			4			1,2,3						
		231			1		2							
		240				1								
		241†				1,2,3								
		242†				1,2,3								
		250		3	4	1	2							
		251		3	4	1	2							
		277†	1	2,3,4										
		278†	1	2,3,4										
		279†	1	2,3,4										
		280		2		1								
		281		2		1								
		293†	1,3			2								
		294†	1,3			2								
		295†	1,3	2										
		296	1	2										
	Vehicle subgroup	*201†*	1					2	*3,4*					*5,6*
		202†	1					2	*5,6*					*3,4*
		204†	2					1	*5,6*					*3,4*
		206†	1					2	*3,4*					*5,6*
		207†	1					2	*3,4*					*5,6*
		232			2		1				*4*	*5*	*3*	
		234			2		1				*4*	*5*	*3*	
		238			1		2				*4*	*3*	*5*	
		252		3		2	1			*5*	*6*	*7*	*4*	
		253		3		2	1			*5*	*6*	*7*	*4*	
		304†	1,2	3					*4*	*5*				
		255								*3*		*2*	*1*	
		309								*1*				

(*Continued*)

Table 2. (Continued)

		Before Craniectomy						After Craniectomy					
	Rat No.	Pentobarbital	Etomidate	Propofol	Alfaxalone/ Alfadolone	Medetomidine	Ketamine	Pentobabital	Etomidate	Propofol	Alfaxalone/ Alfadolone	Medetomidine	Ketamine
Off-target group	*214*†*	4		1,2,3				*5*					*6*
	230			1		2				*4*			
	*233**			2		1		*5*		*3*	*7*	*4*	*6*
	235†			1	2					*3,4*	*5*		
	*236**			1		2		*5*			*3*	*4*	
	303†	1,2	3					*4*	*5*				
	*216**							*2*		*3*			*1*
	*218**							*2*		*3*			*1*
	228									*1*			
	229									*1*			
	248							*5*	*4*	*2*	*1*	*3*	
	249							*5*	*4*	*2*	*1*	*3*	
	270									*1*			*2*
	271							*1*	*2*		*3*	*4*	*5*
	273							*1*	*2*		*3*	*4*	*5*
Lesion group	**237†**			1		2		**7**		**5,6**	**3**	**4**	
	266							**2**	**4**	**3**	**6**	**5**	**1**
	267							**2**	**4**	**3**		**5**	**1**
	268							**3**		**1**	**4**		**2**
	274							**1**	**2**		**3**	**4**	**5**
	275							**3**	**1**	**5**	**2**		**4**
	276							**3**	**1**	**5**	**2**		**4**
	301							**1**	**2**				
	302							**1**	**2**			**3**	
Control group (43 + 13 = 56 rats)		25	18	21	18	18	19						
Catheter-only subgroup (43 rats)		19	13	16	12	12	14						
Vehicle subgroup (13 rats)								*6*	*5*	*5*	*6*	*6*	*5*
Off-target group (15 rats)								***10***	***5***	***10***	***7***	***6***	***7***
Lesion group (9 rats)								**9**	**7**	**6**	**6**	**5**	**6**

The order of the drugs given to each individual rat are indicated by numbers 1 to 7. Rat No. identifies the individual animals and the order in which they were entered into the experiments. Experimental groups indicated by font: standard font indicates control group; italic text indicates control group (vehicle subgroup); bold, italicized text indicates off-target group; bold text indicates lesion group.

*Rats with intentionally off-target lesions. †Rats tested with the same anesthetic agent twice (duplicate) or three times (triplicate) are indicated as such with two or three digits, separated by commas, in a single column. For calculation of lesion effects, averages of duplicate and triplicate values were used.

microinjected with vehicle or ibotenic acid without prior testing. The order of drug delivery was interleaved across experimental animals with avoidance of unintended duplicate tests of the same agent. Group allocation and the order of drug delivery for each rat are detailed in table 2. At least 2 days of rest was allowed between consecutive trials (mean, 6 days). Dose–response curves were constructed for each animal on each drug tested in that animal, plotting anesthesia score against the accumulated drug dose. Curves for each drug were then averaged across rats by experimental group.

To evaluate reproducibility of dose–response curves in individual animals, in a small number with an implanted catheter, but no craniectomy, we ran tests in triplicate for etomidate (three rats), propofol (two rats), alfaxalone/alfadolone (two rats), and ketamine (five rats). Individual trials were separated by at least 3 days, and the first and third trials were separated by 6 to 16 days. The first trial results of each rat were averaged to yield a single dose–response curve representing the rats' first trial on each drug. This was repeated for the second and third drug administration trials.

An additional experiment was carried out to determine whether there might be a prominent difference between male and female rats in the response to GABAergic anesthetics and/or in the effect of lesions of the mesopontine tegmental anesthesia area. For this purpose eight catheterized females (service rats, before craniectomy) were tested for response to iv pentobarbital, and four were tested after on-target placement of mesopontine lesions. Outcomes were compared with dose–response curves of 22 male rats tested before lesioning and 13 with on-target lesions (data for the males are from Minert and Devor[19]). Anesthetic doses were normalized to body weight.

Microinjection

One result of the lesion experiments prompted us to check whether alfaxalone microinjected directly into the mesopontine target induces anesthesia. This was assessed in five naïve (service) rats using two complementary methods described previously in detail. Briefly, using the indwelling cannula method,[16] 500 nl of alfaxalone (10 mg/ml; Alfaxan, Jurox, United Kingdom) was deposited unilaterally in the mesopontine tegmental anesthesia area in awake-behaving rats. The second was the bonus-time method.[18] Here, animals were transiently anesthetized with an iv bolus dose of 1% propofol. Then just before the anticipated time of emergence, 50 to 200 nl of alfaxalone (10 mg/ml) were microinjected into the mesopontine tegmental anesthesia area through a fine glass pipette. We measured whether the microinjected drug would substantially extend the time to emergence from the propofol ("bonus time").

Histology

When experimental observations were completed (mean, 42.6 ± 15.5 days after the first surgery; range, 17 to 59 days), the rats that underwent an intracranial procedure were deeply anesthetized with chloral hydrate and perfused transcardially with 0.9% saline followed by 4% formaldehyde in 0.1 M phosphate buffer (pH 7.3). The brains were removed at least 1 day later, cryoprotected in 30% sucrose in buffer, and cut serially in 50-μm frozen frontal sections. Two series of floating sections were immunolabeled to visualize glial fibrillary acidic protein (a marker of gliosis; anti-glial fibrillary acidic protein; 1:6,000; Millipore, USA, catalog no. AB5804) or NeuN (a nuclear marker of viable neurons; anti-NeuN 1:25,000; Millipore, USA, catalog no. MAB377) using diaminobenzidine as chromogen. Regions of gliosis and neuronal loss were congruent (more details and photomicrographs in the articles by Minert and Devor[19] and Lanir-Azaria *et al.*[22]).

The extent of lesions was evaluated using the Neurolucida system (MBF Bioscience, USA, version 10.51) by an observer blinded to the experimental results, based on glial fibrillary acidic protein-labeled and NeuN-labeled sections spaced at 300 μm. Lesion boundaries were plotted on standard sections of the rat hindbrain[23] and measured planimetrically. Then, counting frames for the rostral and caudal parts of the mesopontine tegmental anesthesia area[16] (fig. 1) were overlaid bilaterally on the lesion plots, and the extent of the lesioned area within the frame was calculated as a fraction of the total frame area. To assess left–right symmetry of lesions, left-sided outlines of the lesion area within the boundaries of the mesopontine tegmental anesthesia area frame were superimposed on mirror-image right-sided outlines, and the area in common was outlined and measured planimetrically.

Finally, in the nine lesion group animals, the percentage of neuronal loss was evaluated in 300 × 300–μm regions in the center of the counting frames by comparing the density of NeuN-immunoreactive nuclei in these rats with corresponding values from four sham-operated service rats in which a glass micropipette was inserted into the mesopontine tegmental target area without injecting vehicle or ibotenic acid. At the end of the study, all animals not prepared for histologic evaluation were euthanized with an overdose of chloral hydrate and confirmation of death.

Statistical Analysis

Sample sizes were based on our previous experience with this study design.[19] No formal statistical power calculation was conducted. Evaluation of drug potency was based on two-tailed, two-sample unequal variance Student's *t* tests comparing the mean dose ± the SD required to reach each of five functional endpoints along the path of anesthetic induction. These were the cumulative drug dose required to reach (1) loss of righting reflex, (2) score of 11 or higher (criterion for anesthesia), (3) score of 14 or higher (criterion for surgical anesthesia), (4) antinociception (immobility in response to noxious pinch), and (5) score of 16 (deep surgical anesthesia). When there was a lesion effect, the dose–response relation shifted rightward in parallel, indicating a close correlation among the five endpoints; fundamentally they all measure the

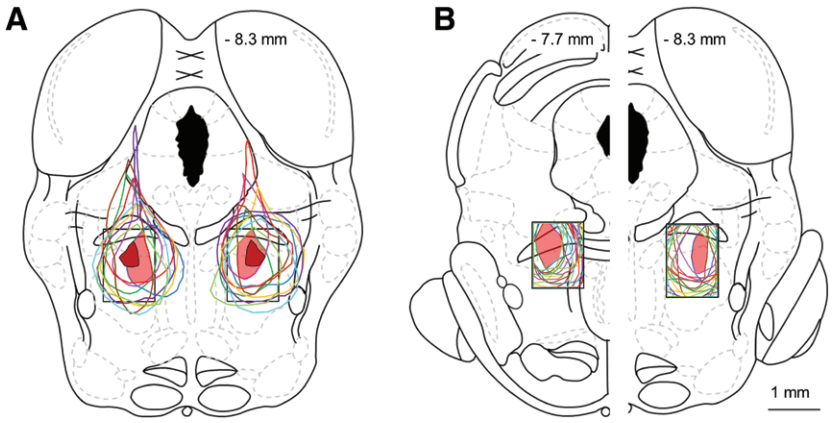

Fig. 1. Location of lesions and left–right symmetry. (*A*) Outlines of the lesion for each of the nine mesopontine tegmental anesthesia area lesion group animals as plotted on tissue sections corresponding to the antero-posterior −8.3 mm plane in the rat brain atlas of Paxinos and Watson.[23] Colors code individual animals, *left* and *right* as indicated below. The area of *pink shading* in the center represents the lesion area common to all nine animals on the corresponding side. The zone of *red shading* represents the area in common among small volume muscimol microinjections (10 and 20 nl) that were effective in maintaining anesthesia (common core) as described by Minert *et al.*[18] Note that the loci of effective lesions and effective microinjections overlap. (*B*) Location of the rostral (*left*, antero-posterior −7.7 mm) and caudal mesopontine tegmental anesthesia area counting frames (*right*, antero-posterior −8.3 mm) used to quantify the extent and left–right symmetry of the lesions. Dimensions of the regions of interest are: 1,000 μm × 1,300 μm and 1,000 μm × 1,500 μm, respectively.[16] Outlines within show the extent of the lesion of each rat that fell within the counting frame (same *color code* as in [*A*]). Outlines on the *left* (antero-posterior −7.7) show left-side lesions superimposed on the mirror image of the right-sided lesions. Correspondingly, outlines on the *right* (antero-posterior −8.3) show right-side lesions superimposed on the mirror image of the left-sided lesions. The areas of *pink shading* in the centers indicate the left–right symmetrical area in common to all nine rats on the rostral and caudal (antero-posterior −7.7 and −8.3) planes. Outline colors indicate rat 237 (*brown*), rat 266 (*pink*), rat 267 (*blue*), rat 268 (*green*), rat 274 (*orange*), rat 275 (*purple*), rat 276 (*red*), rat 301 (*olive*), and rat 302 (*teal*; see table 2).

same thing. For this reason *P* values are provided for each endpoint individually without correction for multiple testing. All statistical comparisons assume that observations are independent of each other and analyzed using between-group tests. When referring to individual endpoints, exact *P* values are given in the text. However, when referring to multiple endpoints and multiple anesthetic agents, inequalities are used ($P < $ or $>$) in the text, with the exact *P* values for each comparison provided in tables 3 and 4. The significance of correlations was tested using the Pearson r coefficient. Both types of statistical tests were run in Excel (Microsoft, USA, version 2010). The criterion for significance was $P < 0.05$. All means are given ± SD. We adhered to the applicable Animal Research: Reporting of *In Vivo* Experiments guidelines (https://www.nc3rs.org.uk/arrive-guidelines; accessed December 4, 2019).

Results

Reliability of the Assessment Protocol

Anesthetic potency varied substantially across agents. However, for each agent tested, the triplicate dose–response curves generated were largely superimposable within individual rats and also across rats ($P > 0.1$ for all agents; fig. 2; exact *P* values are provided in table 3), indicating no effect of repeated testing. Triplicate dose–response curves also matched curves from those control group animals that were given only a single exposure to the agent in question ($P > 0.1$, exact *P* values in table 3). The corresponding data for pentobarbital are given by Minert and Devor.[19] The results from rats given triplicate trials of a given drug (or in a few cases duplicate trials, table 2) were averaged, so that for each rat only a single set of values represented the animal's response to the drug. Hereinafter this average constituted the single dose–response curve for the rat in question. The consistency of dose–response curves lends extra credence to lesion-induced differences.

There was also no effect of drug trials carried out before craniectomy. Specifically, for each agent and experimental group (vehicle group and off-target group; table 2), the results in animals tested for the first time after intracranial microinjection were no different from those that had also been tested before craniectomy and microinjection ($P > 0.1$ for all comparisons; exact *P* values in table 3). Finally, there was no indication that, using our protocol, the order of agent testing affected test results. Thus, for example, the mean dose of propofol required to bring catheterized rats (without craniectomy) to a score of 16 when it was the first agent tested (rats 214, 215, 230, 231, 235, 236, 237, and 238; table 2) was no different than when propofol was tested after ketamine (rats 222, 225, and 226; $P = 0.403$), after

Table 3. Reliability of Protocol for Assessment of Induction Using Different Anesthetic Agents, Based on Two Representative Anesthetic Endpoints: Score of 11 or Higher and Score of 14 or Higher

	Etomidate		Propofol		Alfaxalone/alfadolone		Ketamine	
t Test	Score of 11 or Higher	Score of 14 or Higher	Score of 11 or Higher	Score of 14 or Higher	Score of 11 or Higher	Score of 14 or Higher	Score of 11 or Higher	Score of 14 or Higher
First *vs.* second trial	0.492	1	0.178	0.423	0.423	1	0.862	0.397
First *vs.* third trial	0.561	1	0.423	0.633	1	1	0.809	1
Second *vs.* third trial	1	1	0.506	1	0.423	1	1	0.264
Single exposure *vs.* triplicate trials	0.463	0.436	0.526	0.136	0.533	0.864	0.792	0.975
Rat 255 and 309 *vs.* remainder of vehicle subgroup	0.789	0.413	N/A	N/A	N/A	N/A	N/A	N/A
Rats 214, 230, 233, 235, 236, and 303 *vs.* remainder of off-target group	N/A	N/A	0.864	0.923	0.789	0.496	0.203	0.162

Table entries are *P* values comparing: (1) The dose required to reach each endpoint in control animals (before craniectomy) using pairwise comparisons for each of the agents that was tested in triplicate; (2) comparison of results of triplicate trials with those of all other trials of the same agent (single exposure); and (3) animals tested for the first time after craniectomy *versus* animals also tested before craniectomy.

N/A, no *P* value available because of insufficient group size.

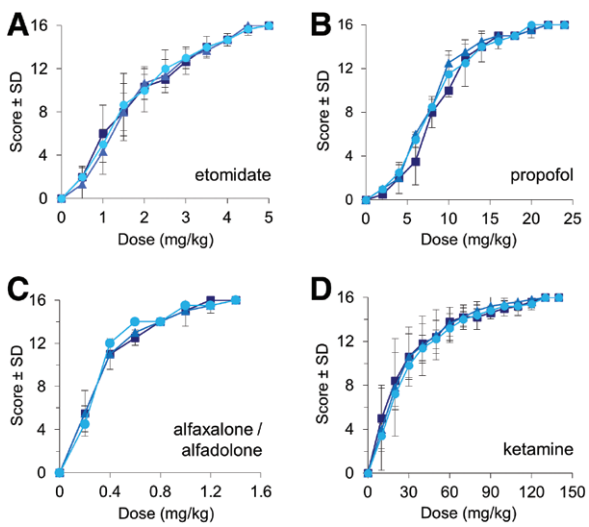

Fig. 2. Dose–response curves varied across anesthetic agents but were highly reproducible upon repeated testing of each individual agent. (*A–D*) Triplicate trials were carried out for etomidate (three rats), propofol (two rats), alfaxalone/alfadolone (two rats), and ketamine (five rats). The data are from the control group animals tested before craniectomy. The plots show the first, second, and third trials of each agent, with values averaged over the number of rats tested with each agent. *Squares* indicate the average of results from the first trials, *triangles* indicate the average of results from the second trials, and *circles* indicate the average of results from the third trials.

medetomidine (rats 232, 233, and 234; $P = 0.114$), or after alfaxalone/alfadolone, medetomidine and etomidate (rats 250 and 251; $P = 0.096$). Having established the reliability of the assessment protocol over repeated test–retest cycles and its robustness when trials of different agents were interleaved, we proceeded to examine effects of brain lesions.

Lesions of the Mesopontine Tegmental Anesthesia Area

The lesioned area in the nine animals that comprised the lesion group all included the "common core." This is the location in the mesopontine tegmental anesthesia area common to all small microinjections of pentobarbital and muscimol (10 or 20 nl) that were effective at maintaining an anesthetic state (fig. 1A).[18] Neuronal loss in the central 300 × 300 μm of the lesion was extensive, with a fall in the density of NeuN immunolabeled neurons from 436.2 ± 43.8 per mm^2 on average in the four nonlesioned service rats examined, to 93.2 ± 72.8 per mm^2 in the nine lesion group animals (78.6% cell loss, $P = 1.3$ E-6). Photomicrographs illustrating such lesions are available in Minert and Devor[19] and Lanir-Azaria *et al.*[22] No lesion was observed in histologic sections of vehicle-microinjected rats, although minor gliosis along the trajectory of the micropipette was seen in some.

We determined previously that bilaterally symmetric mesopontine tegmental lesions reduce the anesthetic potency of systemically delivered pentobarbital, whereas unilateral lesions had no effect.[19] For this reason we focused here on that portion of the lesion that was left–right symmetrical. Lesions that were off-target had size and proportion of cell loss, similar to lesion group rats, but they were displaced from the midline. Damage therefore tended to be medial in counting frames on one side and lateral on the other. Overall, the left–right symmetrical portion of the lesions in the lesion group (n = 9) encompassed 59.5 ± 14.9% of the mesopontine tegmental anesthesia counting frames on average. The corresponding value for off-target animals was only 2.7 ± 8.0% (n = 15, $P = 4.8$ E-07).

Table 4. Comparison of Dose–Response Relations across Groups and Subgroups Based on All Five Anesthetic Endpoints

t Test	Righting	Score of 11 or Higher	Score of 14 or Higher	Antinociception	Score of 16
Etomidate					
Catheter only subgroup *vs.* vehicle subgroup	0.213	0.220	0.169	0.268	0.346
Control group *vs.* lesion group	0.056	0.003	0.003	0.002	0.001
Control group *vs.* off-target group	0.641	0.534	0.892	0.861	0.275
Propofol					
Catheter only subgroup *vs.* vehicle subgroup	0.545	0.711	0.798	0.670	0.201
Control group *vs.* lesion group	0.326	0.018	0.062	0.018	0.010
Control group *vs.* off-target group	0.402	0.813	0.983	0.740	0.663
Alfaxalone/alfadolone					
Catheter only subgroup *vs.* vehicle subgroup	0.008	0.000	0.009	0.002	0.012
Control group *vs.* lesion group	0.054	0.374	0.549	0.629	0.519
Control group *vs.* off-target group	0.984	0.415	0.647	0.635	0.892
Medetomidine					
Catheter only subgroup *vs.* vehicle subgroup	0.506				0.829*
Control group *vs.* lesion group	0.366				0.063*
Control group *vs.* off-target group	0.142				0.769*
Ketamine					
Catheter only subgroup *vs.* vehicle subgroup	0.419	0.180	0.036	0.468	0.729
Control group *vs.* lesion group	0.313	0.336	0.162	0.071	0.039
Control group *vs.* off-target group	0.327	0.989	0.674	0.225	0.005

Table entries are *P* values for the pairwise comparisons indicated.

*Maximal score rather than score of 16.

Mesopontine lesions, both on- and off-target, did not cause coma or sedation, and weight-gain and grooming were normal postoperatively. The sleep–wake cycle persisted, although we know from a previous study that symmetrical on-target lesions cause a degree of insomnia.[19,22]

Effect of Mesopontine Tegmental Anesthesia Area Lesions on Anesthetic Potency

The major finding was that anesthetic potency of some, but not all, of the agents tested was suppressed in a statistically significant manner. No data were missing, declared to be outliers, or otherwise excluded from the analyses.

Control Group. In vehicle-microinjected rats, the dose-response relation for etomidate, propofol, medetomidine, and ketamine were virtually identical to those of rats in the catheter-only subgroup. For each agent, on all five anesthetic endpoints, differences were not statistically significant ($P > 0.1$, exact P values in table 4) except for the ketamine score of 14 or higher, ($P = 0.036$). Because the catheter-only subgroup included data from animals that went on to receive a vehicle microinjection (table 2), we repeated this comparison after excluding their data. The results remained unchanged. We conclude that performing a craniectomy, lowering a probe into the brainstem, and injecting a volume of inert fluid had essentially no effect on anesthetic potency. On these grounds, the results obtained from vehicle-microinjected rats (n = 13) and control rats that did not undergo craniectomy (n = 43) were combined to form a single control group. The data from this group of 56 rats were used to assess changes in the lesion group (n = 9) and the off-target group rats (n = 15). For alfaxalone/alfadolone, the results in the two subgroups did differ because of unusually low intertrial variance. To be safe, results using alfaxalone/alfadolone were compared with the catheter-only subgroup, although the outcome would have been the same had the comparison been with the complete control group.

Lesion Group. Lesioning the mesopontine tegmental anesthesia area caused a statistically significant parallel rightward shift in the dose–response relation for etomidate and propofol for all endpoints ($P < 0.005$; exact P values in table 4) except for loss of righting reflex, for which $P = 0.056$ and $P = 0.326$, respectively, and a score of 14 or higher for propofol ($P = 0.062$; fig. 3). This indicates a lesion-induced loss of efficacy for both agents. No such loss was seen for alfaxalone/alfadolone (except for $P = 0.004$ for score of 11 or higher) or ketamine ($P > 0.05$ on all endpoints; exact P values in table 4). Indeed, for ketamine, there was a small *decrease* in the dose required to achieve the score 16 endpoint ($P = 0.039$). Medetomidine yielded sedation and loss of righting reflex, but because of its weak analgesic action, it did not yield a criterion score for anesthesia or antinociception. A plateau anesthesia score was obtained with medetomidine at a dose of 88 μg/kg iv (score of 7.1 ± 0.3 for the control group). Further increases in dose only marginally increased the score up to the highest dose tested (112 μg/kg iv; score of 7.3 ± 0.5; $P > 0.1$; exact P values in table 4). For this reason, hereinafter we provide data for medetomidine on two endpoints only: loss of righting reflex and

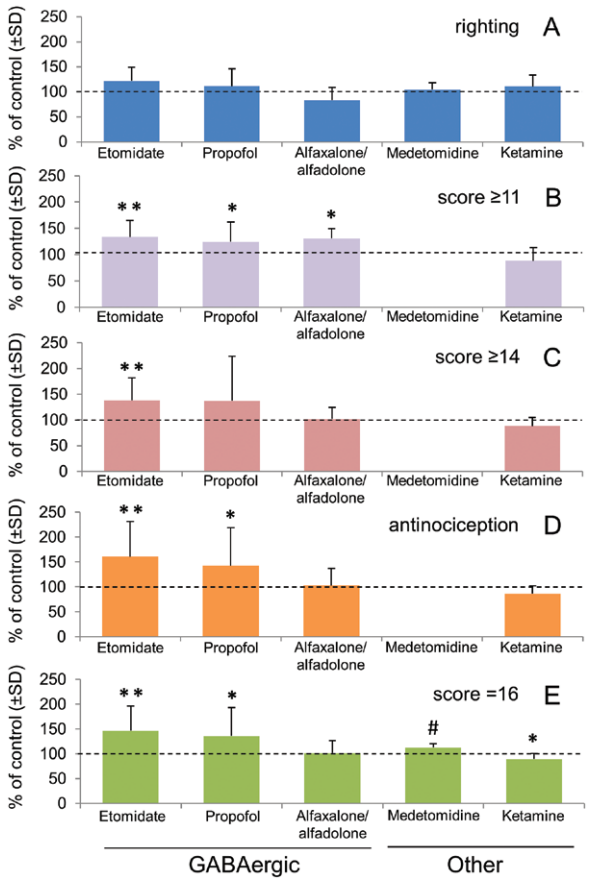

Fig. 3. Mesopontine tegmental anesthesia area lesions reduce sensitivity to some anesthetic agents, but not others. (*A–E*) *Vertical bars* show the agent dose required to reach five different behavioral endpoints of anesthesia in the lesion group animals as a percentage of the dose required in control group animals. Animals with off-target lesions were not included. In (*A*), *righting* refers to loss of righting reflex. The numbers of lesioned and control rats used for each evaluation were as follows: etomidate, 7 lesioned and 18 controls; propofol, 6 lesioned and 21 controls; alfaxalone/alfadolone, 6 lesioned and 12 controls; medetomidine, 5 lesioned and 18 controls; and ketamine, 6 lesioned and 19 controls (also see table 2). Note that the *asterisk* for the score 16 criterion for ketamine indicates an *increase* in potency (*$P < 0.05$; **$P < 0.01$). The corresponding values for pentobarbital for the same 9 lesion group animals compared to 32 controls were as follows: loss of righting reflex (righting), 162 ± 42%; score of 11 or higher, 163 ± 45%; score of 14 or higher, 144 ± 37%; antinociception, 140 ± 29%; and score of 16, 143 ± 41% (for all, $P < 0.01$; extracted from our previously published observations).[19] #For medetomidine, the percentage of change was calculated for the maximal anesthesia score obtained with this agent (score of 7.3 ± 0.5) and not a score of 16. Values in the figure are given as percentages of control animals. Absolute values are provided in Supplemental Digital Content 4 (http://links.lww.com/ALN/C145). GABAergic, γ-aminobutyric acid–mediated.

maximal anesthesia score obtained. Lesions had no effect on the potency of medetomidine on either endpoint ($P > 0.05$; fig. 3; table 4).

The change in response to the anesthetic agents is nicely illustrated in histograms that show the percentages of lesioned and control animals that reached particular anesthetic endpoints as the drug dose was increased (fig. 4). For example, using etomidate, 100% of the control group animals tested (n = 18) reached the maximal anesthesia score of 16 by 5.5 mg/kg iv. The lesion group animals tested with etomidate (n = 7) required 10.5 mg/kg iv before all reached this anesthetic plane (fig. 4A). Our prior results revealed a similar pattern of lesion-induced decrease in potency for (GABAergic) pentobarbital.[19] Dose–response curves comparing sensitivity with pentobarbital in male *versus* female rats (see Materials and Methods) were virtually identical both before and after lesioning ($P > 0.1$). Likewise, the effect of lesioning was the same for males and females ($P < 0.001$ for all endpoints in both sexes; exact P values are given in Supplemental Digital Content 2, http://links.lww.com/ALN/C143; also see Supplemental Digital Content 3, http://links.lww.com/ALN/C144). This justified use of males in the main study.

Off-target Lesion Group. This experimental group comprised rats with histologically verified brainstem injury equivalent to the lesion group, but with little or no bilaterally symmetric involvement of the mesopontine tegmental anesthesia area. These animals showed no statistically significant increase in the dose required to reach any of the anesthetic endpoints compared with control group animals ($P > 0.15$ for all of the agents tested; exact P values in table 4; also see Supplemental Digital Content 4, http://links.lww.com/ALN/C145). Again, for ketamine, the dose required *decreased* for a score of 16 ($P = 0.005$). This outcome emphasizes the importance of accurate placement of mesopontine lesions.

Regression Analysis. Although lesion parameters were the same across animals, there was variation in the completeness, size, and location of the lesions. We exploited this by plotting the response to anesthetics as a function of the extent of the (on-target) lesion. For the reason noted above,[19] we stress the extent of the lesion that is bilaterally symmetric. Results, shown graphically for the anesthesia score of 16 endpoint (deep surgical anesthesia) in figure 5 and for all five endpoints in table 5, showed significant correlations on almost all endpoints for etomidate and propofol, but not for alfaxalone/alfadolone, ketamine, or medetomidine. For completeness we also calculated regression values based on the average size of left- and right-sided lesions without regard to bilateral symmetry. Outcomes were similar in this analysis, but as anticipated coefficients of correlation were somewhat lower for etomidate and propofol. Variation in lesion extent comparing rostral *versus* the caudal frames

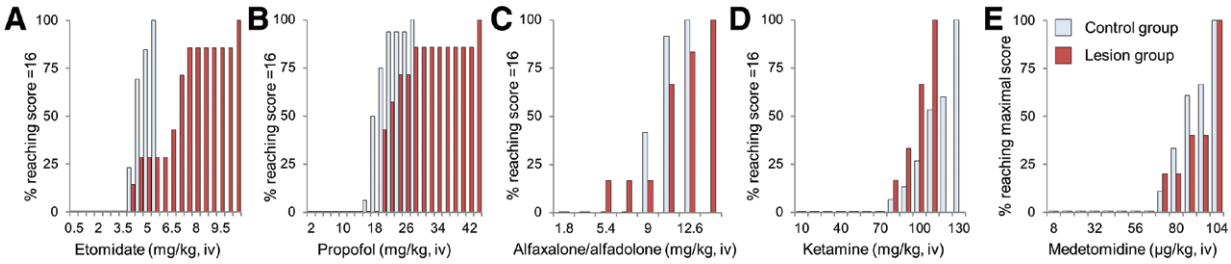

Fig. 4. After lesions of the mesopontine tegmental anesthesia area, higher doses of etomidate and propofol were required to induce anesthesia. (*A–D*) Percentage of animals reaching the score 16 endpoint (criterion for deep surgical anesthesia) as the dose of etomidate, propofol, alfaxalone/alfadolone, and ketamine was gradually increased. (*E*) Same as (*A–D*), but for medetomidine. Because anesthetic depth never reached a score of 16, the effect is given for the highest dose tested (112 μg/kg intravenously [iv]). The number of rats tested for each agent is given in the figure 3 legend (also see table 2).

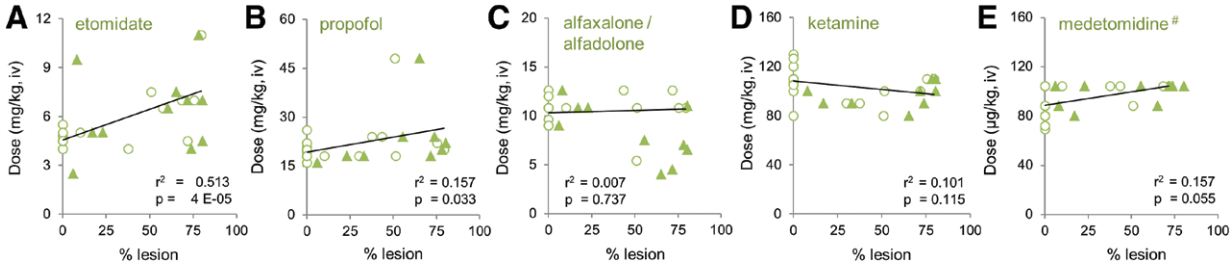

Fig. 5. The dose of etomidate and propofol required to induce general anesthesia depends on the extent of the mesopontine tegmental anesthesia area lesion. This was not so for alfaxalone/alfadolone, ketamine, or medetomidine. (*A–D*) Doses of etomidate, propofol, alfaxalone/alfadolone, and ketamine required to reach a score of 16 (criterion for deep surgical anesthesia) as a function of completeness of the mesopontine tegmental anesthesia area lesion. (*E*) For medetomidine, data are plotted for the maximal score obtained (7.3 ± 0.5) rather than for a score of 16. The *symbols* represent individual rats in the lesion group, those rats in the off-target group in which the lesion encroached at least partially on the mesopontine tegmental anesthesia area, and control group rats. The values for control group animals, all plotted as 0% lesion, include: n = 18 rats for etomidate, alfaxalone/alfadolone, and medetomidine; n = 19 for ketamine; and n = 21 for propofol. Lesioned animal are represented twice: the percentages of lesion for *open circles* indicate the lesioned area that was left–right symmetrical. The *filled triangles* indicate the mean extent of the lesion, averaging rostral and caudal planes, bilaterally. The regression lines plotted and their r^2 and *P* values are based on the symmetrical lesion data (*open circles*). Complete data on the regression analysis are given in table 5.

(fig. 1B) was too small to determine whether there is a functional gradient along the rostrocaudal axis.

Lesion Effect on Emergence from Anesthesia. We reported previously that lesioning the mesopontine tegmental anesthesia area caused a statistically significant increase in the time required for animals anesthetized with pentobarbital to emerge from anesthesia (defined as recovery of the righting reflex).[19] This was also true for systemic etomidate and propofol. The control group animals recovered the righting reflex in 23.6 ± 3.8 and 20.0 ± 4.5 min, respectively, whereas the animals in the lesion group took 36.9 ± 14.8 min for etomidate ($P = 0.030$) and 37.7 ± 9.2 min for propofol ($P = 0.005$). Emergence times for alfaxalone/alfadolone and for ketamine were not significantly altered by on-target lesions (for alfaxalone/alfadolone, 29.7 ± 8.9 min *vs.* 38.0 ± 4.2 min, $P = 0.057$; and for ketamine, 66.6 ± 14.8 min *vs.* 65.0 ± 36.3 min, $P = 0.928$). Off-target lesions had no effect on time to emergence from anesthesia using any of the drugs tested (etomidate, $P = 0.359$; propofol, $P = 0.167$; alfaxalone/alfadolone, $P = 0.677$; and ketamine, $P = 0.135$).

Microinjection. Although the neurosteroids alfaxalone and alfadolone are believed to act *via* $GABA_A$ receptors,[24–26] on-target mesopontine lesions failed to affect their anesthetic potency upon systemic administration, unlike the other GABAergic agents tested (pentobarbital, etomidate, and propofol). In light of this discrepancy, we checked the effect of microinjecting one component, alfaxalone, directly into the mesopontine tegmental anesthesia area. Seven trials were carried out using the indwelling cannula method, none of which yielded a criterion change in anesthesia score (three rats, all with scores of 1 or lower). In addition, three trials were carried out using the bonus time method.

Table 5. Correlation between the Extent of Lesion and Dose Required to Reach Anesthetic Endpoint

	Etomidate 26 rats		Propofol 29 rats		Alfaxalone/ Alfadolone 25 rats		Medetomidine 24 rats		Ketamine 26 rats	
Anesthetic Endpoint	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
Righting	0.188	0.027	0.052	0.232	0.151	0.055	0.028	0.4337	0.052	0.262
Score of 11 or higher	0.457	2E-04	0.214	0.012	0.042	0.328			0.021	0.483
Score of 14 or higher	0.418	4E-04	0.111	0.078	0.005	0.727			0.058	0.237
Antinociception	0.460	1E-04	0.153	0.036	0.009	0.654			0.109	0.1
Score of 16	0.513	4E-05	0.151	0.038	0.011	0.624	0.157*	0.055*	0.101	0.115

The r^2 and *P* values are for the correlation between the extent of the symmetrical mesopontine tegmental anesthesia area lesion and the dose needed to reach five standard anesthetic endpoints. Number of rats includes control animals with 0% lesion.

*Maximal score rather than score of 16.

Here too, none yielded a statistically significant prolongation of the duration of anesthesia (two rats, mean bonus time of 5 min).[18] In both experimental paradigms, on-target deposition of the alfaxalone was verified histologically and/or by documenting a statistically significant effect of microinjecting muscimol at the same location.

Discussion

Lesioning the mesopontine tegmental anesthesia area renders rats relatively insensitive to induction by the GABAergic agents etomidate and propofol, as well as pentobarbital.[19] Although drug potency was never reduced by more than twofold, the change was statistically significant and clinically meaningful, bringing the effective dose, for pentobarbital and propofol at least, close to levels lethal in intact animals.[27] Effects were proportional to the extent of the lesion. Microinjected vehicle and off-target lesions had little effect. Surprisingly, no lesion effect was found for alfaxalone/alfadolone, neurosteroids considered to be GABAergic.[24–26] Potency of the non-GABAergic anesthetics ketamine and medetomidine[28,29] was also largely unaffected. These observations (1) affirm a key role for the mesopontine nucleus in transitioning from wakefulness to the anesthetic state, (2) implicate dedicated axonal pathways as mediators of brain-state switching, and (3) suggest selective sensitivity of target mesopontine neurons to particular GABAergic agents.

Lesioning hindbrain and forebrain arousal/anesthesia nuclei is nearly always neutral or *increases* agent potency.[12,30–32] In contrast, we observed *reduced* potency. The ventrolateral preoptic nucleus, a master driver of sleep, may be another exception. Lesions here reduce sensitivity to dexmedetomidine but increase sensitivity to isoflurane.[33] Other potential exceptions are the lateral habenula[34] and the supraoptic nucleus,[35] where functional suppression reduces the hypnotic effect of particular anesthetics given systemically.

Receptor Selectivity

The obvious common denominator of agents affected by on-target lesions is GABAergic pharmacology; all are positive allosteric modulators of $GABA_A$ receptors at the doses used.[26,36] On these and other grounds, we hypothesize that the mesopontine tegmental anesthesia area functions as a sensor of exogenous and presumably also endogenous $GABA_A$ receptor agonists, driving a bistable (flip-flop) switch for transitioning between wakefulness and unconsciousness.[8,37,38] In this regard failure of lesions to affect the potency of the neurosteroids alfaxalone/alfadolone, as well as failure of directly microinjected alfaxalone to induce or maintain anesthesia, is puzzling. Note, however, that a growing literature stresses the heterogeneity of $GABA_A$ receptor isoforms. Tonic, extrasynaptic receptors that mostly employ the δ-subunit, for example, respond to GABA and other direct agonists at much lower concentrations than phasic, synaptic isoforms containing the γ_2-subunit, and they are virtually unresponsive to benzodiazepines.[3,25,36,39–44] To explain the failure of our lesions to affect the anesthetic potency of alfaxalone and alfadolone, we tentatively suggest that those mesopontine neurons that are responsible for brain-state switching express isoforms unresponsive to these neurosteroids, although not necessarily unresponsive to other neurosteroids. Their ability to induce anesthesia, like that of ketamine and medetomidine, might reflect actions outside of the mesopontine tegmentum.

Anesthetic Induction by Dedicated Axonal Pathways

Neurons expressing $GABA_A$ receptors are ubiquitous in the CNS and are accessible to circulating GABAergic anesthetics.[1–3,5] Thus, cortical, spinal, and other neurons in our lesion group animals would have continued to be exposed to etomidate and propofol (and pentobarbital)[19] at concentrations proven adequate for anesthetic induction in control group animals. The fact that the animals nonetheless remained responsive to pinch suggests that, absent the mesopontine

tegmental anesthesia area, widespread CNS exposure to these agents is *not* sufficient to induce anesthesia. The failure of decortication to alter minimum alveolar concentration for isoflurane or to induce a state resembling anesthesia is consistent with this observation.[31,45–47] In contrast, as we have shown, selective exposure of a small cluster of mesopontine tegmental neurons to anesthetics *is* sufficient. This suggests that the primary locus of GABAergic drug action in systemic-induced anesthesia is within the mesopontine tegmentum, in rodents at least. The tiny doses effective upon microinjection and the rapid effect kinetics preclude widespread redistribution of the agents themselves. Engagement of the loci where the functional components of anesthesia are actually executed, cortex, spinal cord, and elsewhere, must therefore be mediated by dedicated axonal pathways.[17,38,48]

Immobility: Mesopontine Tegmentum or Spinal Cord?

Where in the CNS do GABAergic anesthetics act? Antognini *et al.*[1] provided evidence that immobility upon noxious stimulation (antinociception) is realized in the spinal cord. Specifically, using a vascular bypass procedure in goats, they showed that selective exposure of the brain to anesthetics (including GABAergics) induces immobility, but when both brain and spinal cord are exposed, immobility occurs at concentrations lower by half (thiopental, 42 ± 6 *vs.* 20 ± 10 μg/ml).[49] This suggests an exclusively spinal mechanism under normal clinical conditions or at least a mechanism predominantly spinal. The 20 ± 10 μg/ml concentration, after all, was effective when delivered to the whole body but did not yield immobility when delivered selectively to the brain. This conclusion, however, is incompatible with our observation that in the rat, targeted mesopontine microinjection induces deep anesthesia, including immobility, with no drug reaching the cord. Thus, in addition to being *sufficient*, exposure of mesopontine neurons to anesthetics may be *necessary* for anesthetic induction on the grounds that in lesion group animals, normally effective systemic doses lost their ability to induce immobility.

A species difference, goat *versus* rat, might be invoked to reconcile these conflicting results. However, there are more parsimonious explanations. For example, the bypass procedure might have compromised blood flow to the brainstem in the goats such that more thiopental was needed in the blood to reach an adequate concentration in the mesopontine tegmentum. Likewise, sensitivity of the local neurons to the anesthetic agent may have declined because of impaired oxygen or nutrient supply. It is also possible that in the bypass mode a larger fraction of the thiopental delivered became bound to plasma proteins and hence inaccessible to membrane receptors. Under each of these scenarios, it is possible that the entire immobilizing effect in goats might have been due to anesthetics acting in the brainstem. No studies are available showing that 20 ± 10 μg/ml thiopental delivered exclusively to the spinal cord in goats is sufficient to induce immobility. Likewise, none show that delivery of this concentration exclusively to the mesopontine tegmentum is not sufficient.

Another approach to reconciling the data from goats and rats is synergy. As noted, Antognini *et al.*[1] documented in goats that 42 ± 6 μg/ml thiopental delivered selectively to the head induces immobility. Thus, at this concentration, anesthetic action in the brain alone is sufficient to generate immobility, presumably mediated by secondary recruitment of descending bulbospinal pathways.[15,50] We propose that in the clinical setting (goats, rats, and humans), where drugs access both the cord and the brainstem, immobility occurs at relatively low concentrations because of synergistic action at both loci. Delivered selectively to the brain higher concentrations are needed to generate more powerful descending inhibition. Correspondingly, when the mesopontine nucleus is lesioned, the systemic drug dose must be increased to compensate for the reduction in descending inhibition. The now increased blood levels of the drug may act in the brainstem, spinal cord, and/or elsewhere in the CNS. The synergy hypothesis makes a testable prediction. Specifically, selective delivery of GABAergic agents to the mesopontine tegmentum at concentrations actually achieved at this locus after delivery of clinically effective doses to the systemic circulation ought to be insufficient to produce immobility. An additional boost is required.

Off-target Effects of Systemically Administered Anesthetics

When etomidate, propofol, or pentobarbital were delivered systemically at high doses, surgical anesthesia was induced despite on-target mesopontine lesions. There are several potential reasons for this. Perhaps at high concentrations the drugs recruit latent, relatively insensitive mesopontine neurons that survived the lesion. Lacking a molecular marker of the effective neurons, we do not know their precise number or location, and at present we lack tools to lesion them all precisely. Alternatively, at high systemic doses antinociceptive actions in the spinal cord might be enhanced, or secondary anesthetic-sensitive nodes elsewhere in the brain might be engaged. Finally, at elevated concentrations anesthesia might be induced in lesioned animals by nonspecific, generalized CNS inhibition (the global suppression, or "wet-blanket," hypothesis).[8]

Various brain phenomena, most notably enhanced electroencephalographic power in the δ-band and reduced effective cortical connectivity, herald anesthesia-induced loss of consciousness and immobility, and perhaps cause them.[51,52] If so, it is important to establish whether and how these neural correlates are implemented by axonal pathways ascending and/or descending from the mesopontine tegmentum.[38] Even well known neural correlates of anesthetic induction, of course, may not actually be causative. Some, for example, might constitute incidental, functionally neutral side effects of off-target neural suppression, whereas

others might reflect unwanted off-target effects such as respiratory depression and altered thermoregulation.

Conclusions

We conclude that at clinically relevant doses at least some GABAergic anesthetics act within the mesopontine tegmental anesthesia area to trigger secondary recruitment of dedicated executive pathways that implement anesthesia. An unavoidable implication is that anesthetics mobilize preexisting, adaptive circuitry that was designed evolutionarily to promote orderly transition between wakefulness and unconsciousness. This circuitry likely evolved in the context of natural events that implement loss of consciousness such as sleep, metabolic stress, syncope, threat by predators, and others.[22,53,54] The greater ease of arousing from sleep than arousing from anesthesia most likely reflects the *in vivo* pharmacokinetics of the corresponding chemical mediators: neurotransmitters and neuromodulators on the one hand and exogenous anesthetic compounds on the other. In this sense, anesthesia resembles morphine-induced analgesia where a synthetic compound substitutes for morphine-like neurotransmitters in endogenous pain-control circuitry.[55] From this viewpoint, anesthesia is a medical invention whereby an evolutionarily adaptive network dedicated to executing brain-state transitions is pharmacologically co-opted to permit pain-free surgery.

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Competing Interests

The authors declare no competing interests.

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