Norepinephrine Infusion into Nucleus Basalis Elicits Microarousal in Desflurane-anesthetized Rats

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ABSTRACT

Background: The nucleus basalis of Meynert of the basal forebrain has been implicated in the regulation of the state of consciousness across normal sleep-wake cycles. Its role in the modulation of general anesthesia was investigated.

Methods: Rats were chronically implanted with bilateral infusion cannulae in the nucleus basalis of Meynert and epidural electrodes to record the electroencephalogram in frontal and visual cortices. Animals were anesthetized with desflurane at a concentration required for the loss of righting reflex (4.6 ± 0.5%). Norepinephrine (17.8 nmol) or artificial cerebrospinal fluid was infused at 0.2 μl/min (1 μl total). Behavioral response to infusion was measured by scoring the orofacial, limb, and head movements, and postural changes.

Results: Behavioral responses were higher after norepinephrine (2.1 ± 1) than artificial cerebrospinal fluid (0.63 ± 0.8) infusion ($P < 0.01$, Student t test). Responses were brief (1–2 min), repetitive, and more frequent after norepinephrine infusion ($P < 0.0001$, chi-square test). Electroencephalogram delta power decreased after norepinephrine in frontal (70 ± 7%) but not in visual cortex ($P < 0.05$, Student t test). Simultaneously, electroencephalogram cross-approximate entropy between frontal and visual cortices increased from 3.17 ± 0.56 to 3.85 ± 0.29 after norepinephrine infusion ($P < 0.01$, Student t test). Behavioral activation was predictable by the decrease in frontal delta power (logistic regression, $P < 0.05$).

Conclusions: Norepinephrine infusion into the nucleus basalis of Meynert can modulate anesthetic depth presumably by ascending activation of the cortex. The transient nature of the responses suggests a similarity with microarousals normally observed during natural sleep, and may imply a mechanism for transient awareness under light anesthesia.

What We Already Know about This Topic

- Endogenous sleep and arousal pathways are implicated in anesthetic-induced loss of consciousness
- The role of cholinergic innervation of the cerebral cortex by the nucleus basalis, an ascending arousal pathway, in anesthesia is unclear

What This Article Tells Us That Is New

- Microinfusion of the excitatory transmitter norepinephrine into the nucleus basalis of rats produced transient behavioral and electroencephalographic activation during desflurane anesthesia
- Ascending cholinergic arousal pathways might be involved in modulating the depth of general anesthesia

THE mechanisms by which anesthetic agents produce loss of consciousness (LOC) remain a mystery. In addition to their direct effects on the thalamus and cerebral cortex,[1–6] anesthetic agents may encourage LOC through an interaction with the ascending arousal system.[7–14] The ascending arousal system consists of parallel pathways that use cholinergic and various aminergic neurotransmitters that function to establish, maintain, and modulate cortical and behavioral arousal.[15–17] They are grouped into ventral and dorsal pathways; the ventral pathways run through the hypothalamus and the basal forebrain, and the dorsal pathways travel through the thalamus.[16]

The cholinergic component of the ascending arousal system, in particular, has been implicated in the regulation of states of consciousness across normal sleep-wake cycles and may also be relevant for general anesthesia. Acetylcholine concentration in the cortex is generally increased during periods of wakefulness and rapid eye movement sleep.[18–23] Cholinergic activation *via* intracerebroventricular infusions of the acetylcholinesterase inhibitor neostigmine or the muscarinic agonist oxotremorine reverses the electroencephalo-

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graphic and behavioral depressant effect of isoflurane.[10] Propofol or sevoflurane-induced LOC can be reversed *via* intravenous administration of physostigmine.[24,25] Microinfusion of nicotine into the thalamic central medial intralaminar nucleus can also reverse sevoflurane anesthesia in rats.[26]

A major source of cholinergic innervation to the cerebral cortex and limbic system is the basal forebrain, more specifically the nucleus basalis of Meynert (NBM).[16,27–30] Heightened basal forebrain activity is correlated with an increase in acetylcholine concentration in the cortex.[18,20,29] Conversely, inactivation of the basal forebrain by local administration of γ-aminobutyric $acid_A$ agonists reduces acetylcholine release and cortical acetylcholine turnover.[31] NBM activity is controlled by inputs from brainstem and hypothalamic arousal systems.[16,32]

Despite the significance of NBM in arousal state regulation, few studies have examined the role of the NBM in modulation of the anesthetic state. It has been shown that the anesthetic potency of propofol is potentiated after cholinergic lesion of the NBM.[33] Microinfusion of histamine into the NBM facilitated electroencephalographic arousal and emergence from isoflurane.[34] None of these studies examined whether pharmacologic manipulation of the NBM can reverse LOC during continued desflurane administration. Such an investigation could contribute to the growing body of evidence for the role of sleep-wake regulating brain systems in the modulation of the anesthetic state.[35,36]

The excitatory neurotransmitter norepinephrine is a key modulator of the ascending arousal system,[16,35] and its intracerebral concentration is reduced by anesthetic agents.[35,37] We hypothesized that microinfusion of norepinephrine into the NBM would facilitate electroencephalographic and behavioral arousal during steady-state desflurane anesthesia. To test this hypothesis, we microinjected norepinephrine at a dose comparable with those previously found effective in modulating natural sleep and electrocortical activity.[22,38,39] To represent both motor and sensory functionality, the electroencephalogram was recorded from the frontal and visual cortex, respectively. Behavioral arousal was scored based on orofacial and gross motor movements. At a constant hypnotic concentration of desflurane, norepinephrine microinfusion into the NBM produced transient episodes of behavioral and electroencephalographic activation that resembled microarousals previously observed during natural sleep.[40] These results suggest that the NBM may play a role in the modulation of the depth of general anesthesia.

Materials and Methods

Animals

All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin (Milwaukee, Wisconsin). All procedures were in conformance with the Guiding Principles in the Care and Use of Animals of the American Physiologic Society and were in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996).

Experiments were performed on 11 adult (250–360 g), male, Sprague-Dawley rats (Harlan Laboratories, Madison, WI). All animals were housed in a reverse light-dark cycle room for at least 10 days before surgical implantation, and remained there for the duration of the experiment. Food and water access was *ad libitum*.

Surgical Preparation

Aseptic technique was used during surgical preparation. Animals were anesthetized, through spontaneous breathing, with 1.9 ± 0.2% isoflurane, vaporized into a mixture of 30% O_2, 70% N_2 and delivered at a flow rate of 5 l/min. Anesthesia was distributed through a gas anesthesia mask (Model 929-B Rat Gas Anesthesia Head Holder, David Kopf Instruments, Tujunga, CA). Anesthetic concentration was monitored (POET IQ2 monitor; Criticare Systems, Inc., Waukesha, WI) through a sampling line connected to the anesthesia mask. Core body temperature was rectally monitored (model 73A, YSI, Yellow Springs, OH) and maintained at 37°C with a thermostat-controlled, electric heating pad (TC-1000, CWE Inc., Ardmore, PA).

To prepare the animal for surgery, Betadine (VWR, Radnor, PA) and alcohol were repeatedly applied to the dorsal surface of the head. Sterile 0.5% bupivacaine was administered subcutaneously for local anesthesia. A midline incision was made; the skin and connective tissue were reflected laterally to reveal the cranium. Hydrogen peroxide (in some cases a cautery) was used to stop any bleeding.

For electroencephalogram recording, two pairs of epidural, stainless steel, screw electrodes (Plastics One Inc., Roanoke, VA) were implanted through burr holes in the cranium. One pair of electrodes was located in the frontal cortex (4.5 mm anterior, 2.5 mm lateral, from bregma), and the other pair in the visual cortex (8.3 mm posterior, 2.5 mm lateral, from bregma). Lead wires from the electrodes resided within a six-channel, molded plastic, electrode pedestal (Plastics One Inc.). For infusion of agents into the NBM, each rat was outfitted with two cannulae (center-to-center distance = 5 mm; length = 5 mm) (Plastics One Inc.) that were lowered into the NBM bihemispherically (1.3 mm posterior, 2.5 mm lateral, −5 mm vertical, from bregma). One additional screw was placed behind λ to further anchor the skullcap to the cranium.

The electrode and cannula assembly was secured in place with a gentamicin-enriched bone cement (Palacos R&G, Zimmer Orthopaedic Surgical Products, Dover, OH) and cerebond skull adhesive (Leica Microsystems, Bannockburn, IL). Before removal from the stereotaxic frame, a dummy cannula (length = 7 mm) (Plastics One Inc.) was lowered into the guide cannula. Dust caps were then threaded onto the electrode pedestal and cannula to prevent dirt and debris from causing interference.

The analgesic carprofen (5 mg/kg subcutaneously once daily) and the antibiotic enrofloxacin (10 mg/kg subcutaneously once daily) were administered postoperatively for 2 and 7 days, respectively. Animals were housed individually to reduce the chance for removal of the skullcap.

Drug Preparation and Administration

The norepinephrine (L-(−)-norepinephrine (+)-bitartrate salt monohydrate, Sigma–Aldrich, St. Louis, MO) solution was prepared on the experimental day; it was dissolved in 4 ml artificial cerebrospinal fluid (aCSF) (Harvard Apparatus, Holliston, MA). Drug concentrations used by Metherate *et al.* and Cape and Jones[22,38] served as guides during our pilot experiments (data not reported here). The efficacious dose, defined as the minimum necessary to elicit behavioral arousal, of the drug was determined by injecting norepinephrine at doses of 6, 12, 17.8, 30, and 36 nmol in three pilot rats (did not participate in the study). Higher doses of norepinephrine produced detrimental side effects (abnormal gait, seizure activity, and mortality) due to its toxicity at high concentrations. A dose of 17.8 nmol (6 μg in 1 μl total dose, corresponding to a concentration of 17.8 mM) of norepinephrine was selected for a consistent arousal effect. Two 2-ml glass syringes were outfitted with 23-gauge needles and PE50 (Becton, Dickinson and Company, Franklin Lakes, NJ) tubing; each syringe was connected to one side of the bilateral cannula. The syringes were placed onto an infusion pump (Harvard 22; Harvard Apparatus) to simultaneously drive the microinfusion of norepinephrine or aCSF into each side of the cannula. The drug was flushed through the PE50 tubing before insertion of the infusion cannulae (depth = 7 mm) into the guide cannulae. The drug and vehicle were infused at a rate of 0.2 μl/min for 5 min (total infused volume = 1 μl). Previous estimates reveal that drug diffusion radiates approximately 1 mm from the tip of the infusion cannula at an infusion rate of 0.1 μl/min and injected volume of 0.5 μl[38] to each side of the NBM.

The experimental design was such that each animal would be treated once with either norepinephrine or aCSF on separate days. The order of treatment presentation was randomized, and at least 4 days elapsed before administration of a different treatment. One animal failed to complete all treatment groups because of euthanasia due to removal of the skullcap.

All 11 rats used in the study received norepinephrine infusion. Vehicle was delivered to 10 rats. Animals were exposed to desflurane on experimental days only. A separate sham group of four animals completed the study.

Experimental Protocol

Testing commenced no earlier than 7–10 days postoperatively. On the experimental day, rats were placed into a custom-built, transparent, Plexiglas anesthesia experimental box (46 cm × 23.5 cm × 23 cm). The animal retained the ability to breathe spontaneously throughout the experiment. Desflurane and O_2 (30%) were delivered at a flow rate of 5 l/min and carefully monitored (POET IQ2; Criticare Systems, Inc., Waukesha, WI), and rat body temperature was controlled at 37°C throughout the duration of the experiment. Of particular interest was the point at which LOC occurs. In each rat, the desflurane concentration sufficient for loss of righting reflex (LORR) was determined. LORR is widely used as a surrogate for LOC in rats.[35] LORR was assessed once, before the onset of data collection, by tilting the box at 30° and scored as being present (purposeful attempt to right itself) or absent (lack of an attempt to right itself); the desflurane concentration (4.6 ± 0.5%) at which LORR was achieved remained constant throughout the experiment. This concentration did not change significantly over repeated desflurane exposures.

The experimental protocol is illustrated in figure 1. After establishing the desflurane concentration sufficient for LORR, the rats were allowed a 20-min equilibration time. Electroencephalographic activity and behavioral responses (*via* video recording) were acquired throughout the experiment. The experiment commenced after the equilibration period by acquiring spontaneous resting electroencephalogram and video for 20 min. The infusion cannulae were then manually inserted into the guide cannula, and the gases within the chamber were allowed to equilibrate for 20 min (due to any disturbance to the anesthetic environment that insertion of the infusion cannula may have caused). At the end of the equilibration period, the experiment resumed with a 20-min recording of spontaneous activity. This was followed by the infusion run; it lasted for a total of 10 min, with infusion alternating on and off every other minute. Therefore, total duration of infusion was 5 min at 0.2 μl/min (total infused volume = 1.0 μl). Spontaneous neural and behavioral activity was then acquired for another 60 min.

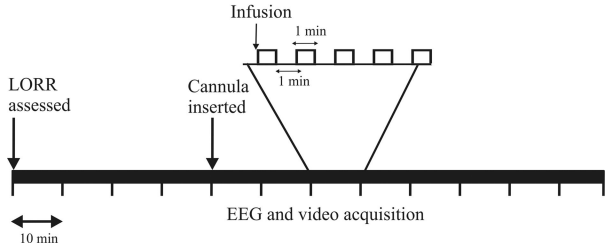

Fig. 1. Experiment timeline. Before the onset of data acquisition the desflurane concentration sufficient for the loss of righting reflex was tested in each rat; this concentration was held constant throughout the rest of the experiment. Spontaneous electroencephalographic and behavioral responses were recorded throughout the experiment. Infusion cannulae were inserted after 20 min of baseline recordings. Drugs were infused in five consecutive 1-min blocks over the course of 10 min (start of infusion, *vertical arrow*). Electroencephalogram recording continued for 1 h after completion of the infusion sequence. The total duration of each experiment was approximately 120 min. LORR = loss of righting reflex; EEG = electroencephalogram.

Behavioral Assessment

Animals were scored on the presence or absence of behavioral response to drug infusion. Behavioral response was assigned a score from 0–4 (0 = no movement; 1 = whisker movement, chewing; 2 = sporadic, purposeful limb and head movement; 3 = sporadic crawling/walking; 4 = fully awake) by the experimenter (was not blinded to the drug treatment). However, behavioral responses were continuously recorded throughout the experimental session to objectively assign a score. A responsive rat was defined as one that achieved a score of at least 2.

Data Acquisition

Electroencephalographic signals recorded from the two regions were analog band-pass filtered at 1–1000 Hz, analog notch filtered at 60 Hz, and digitally sampled at 2,500 Hz using the WINDAQ data acquisition software (DATAQ Instruments, Akron, OH). The data were later downsampled to 500 Hz and 200 Hz for the ensuing spectral and cross-approximate entropy analyses, respectively.

Behavior, in addition to being scored, was recorded using a video acquisition device (Fire-iTM Digital Camera, Unibrain Inc., San Ramon, CA), situated outside of the experimental box. To allow for valid comparisons and analysis between cortical activity and behavioral response(s), the video file recordings were synchronized to electroencephalographic acquisition by sending a brief pulse of light *via* a light emitting diode inside the anesthesia chamber; it was invisible to the rat.

Histologic Verification

At the end of experimental testing, cannulae placement and drug spread was confirmed histologically with Fast Green dye (F7258, Sigma–Aldrich) on chemically fixed coronal sections. The rats were cardioperfused with 300 ml 0.9% saline, followed by 250 ml 4% paraformaldehyde solution, through the heart ventricle. The brains were then harvested and stored in the paraformaldehyde solution for 24 h, and subsequently transferred to 0.01 M phosphate-buffered saline (pH 7.4). The 80 μm-thick coronal brain sections were cut by a vibratome (Vibratome Series 3000 Plus, Ted Pella, Inc., Redding, CA). Brain slices were stained with cresyl violet Nissl and subssequently imaged, using a Nikon Eclipse E600 (Nikon Inc., Melville, NY) microscope, to visualize the location of the cannula and infusion sites. Only one of the non-responding rats revealed incorrect cannula placement.

Data Analysis and Statistics

For the power spectra analyses, preinfusion electroencephalographic data were chosen at a consistent time point across all animals and all treatment groups; they were obtained between 5–10 min of the initial spontaneous recording. These segments were 60 s in duration. Postinfusion data were carefully picked such that these segments were free from motion artifact, 30 s in duration, and coincided, as much as possible, with behavioral responses, as obtained by video recordings. Motion artifacts were large and clearly visible on the raw electroencephalographic trace. For cross-approximate entropy analyses, 10 min of preinfusion and postinfusion data were used. The preinfusion electroencephalographic data were chosen at a consistent time point across animals and treatment groups. The postinfusion data were chosen such that there were several behavioral arousals within a 10-min segment of data.

Electroencephalogram power spectra were calculated from segments of data using the Welch spectral estimation method with a 250-point window and 90% overlap. To reduce the effect of spectral leakage, a Hanning window was used. Band powers (δ = 2–4 Hz, θ = 4–7 Hz, α = 8–12 Hz, β = 12–30 Hz, low-γ [L-γ] = 30–50 Hz, and high-γ [H-γ] = 70–140 Hz) were obtained from the spectra by averaging signal power in the respective frequency ranges. To estimate regional interactions from the electroencephalogram, cross-approximate entropy was computed as detailed in previous studies.[10,41] Cross-approximate entropy is a nonlinear measure of the statistical dissimilarity of signals measured at two remote locations; in this case, in the frontal and visual cortices. Cross-approximate entropy was derived for consecutive 2-s epochs and then averaged within each of the 10-min data segments.

Data from all 11 rats were included in the statistical analyses. The data were tested for normality using the Shapiro-Wilk test, which yielded no reason to reject the normality assumption. The effect of norepinephrine microinfusion into the NBM on electroencephalogram band powers and behavioral score was first tested with repeated measures multivariate ANOVA, with the time (pre *vs.* post), treatment (norepinephrine and aCSF), and brain region as independent variables, rat as the subject (random) variable, and band power and behavioral score as dependent variables. To determine whether the magnitude of the change in delta power was different among the treatments, repeated measures ANOVA was used with treatment (norepinephrine *vs.* aCSF) and brain region (frontal *vs.* visual) as within-factors, rat was the subject variable, and the change in delta power (preinfusion subtracted from postinfusion) as the dependent variable. A chi-square test was performed on the behavioral scores to determine whether the responsive and unresponsive rats were distributed differently across the treatments. A Student *t* test was used to determine whether the magnitude of the change in delta power within the frontal cortex was different among the treatments. To explore if a decrease in delta power could predict the behavioral response, logistic regression was performed. The preinfusion *versus* postinfusion difference in delta power and treatment (norepinephrine *vs.* aCSF) were independent variables, and the behavioral response was the dependent variable.

The effect of norepinephrine microinfusion into the NBM on cross-approximate entropy was first tested with repeated measures ANOVA, with the time (pre *vs.* post), and

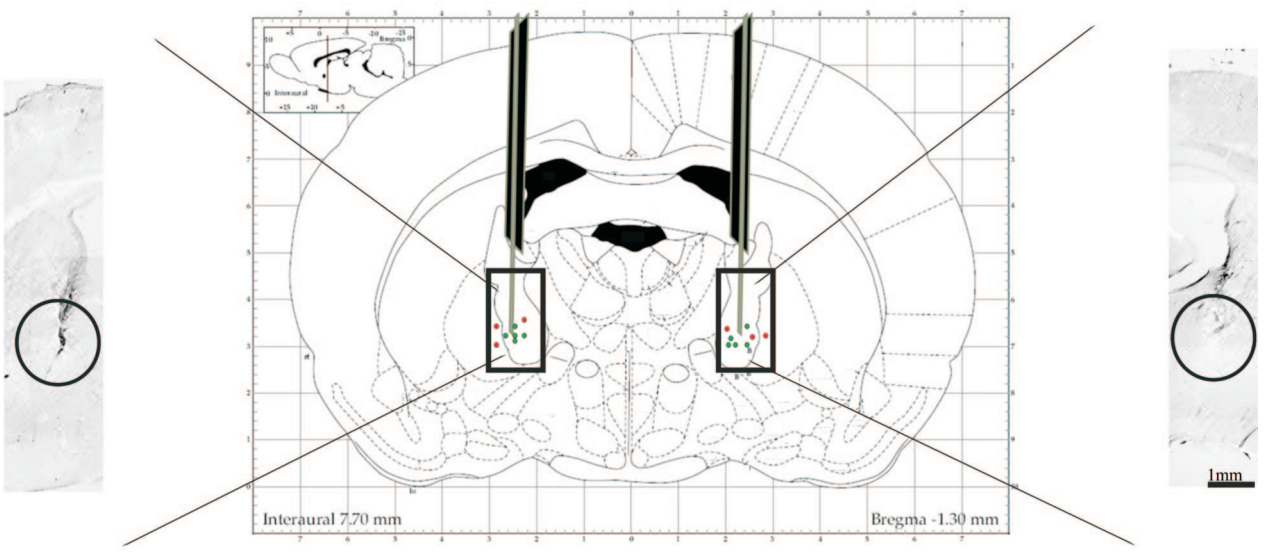

Fig. 2. Histologic verification of cannula placement. Schematic of the infusion cannulae overlaid, with permission, on a stereotaxic drawing from the Paxinos[42] rat brain atlas. This figure was published in The Rat Brain in Stereotaxic Coordinates, 4th Edition, Paxinos G, Watson C, Copyright Academic Press (1998). Used with permission. *Thick solid bars* represent the guide cannulae, *light bars* indicate the internal infusion cannulae. *Colored closed circles* show the infusion location (*green circled regions* = responsive, *red circled regions* = unresponsive). The cannula tracts terminating within the nucleus basalis of Meynert are displayed in the histologic figures. *Open circles* show the diffusion radius.

treatment (norepinephrine *vs.* aCSF) as independent variables, rat as the subject (random) variable, and cross-approximate entropy as the dependent variable. Student *t* test was then used to check for a significant difference in cross-approximate entropy changes (preinfusion subtracted from postinfusion) between norepinephrine and aCSF treatment.

All electroencephalographic data were analyzed using custom scripts in MATLAB version 7.3.0 (MathWorks Inc., Natick, MA). Statistical analyses were performed using NCSS 2007 (NCSS, Kaysville UT). All analyses were two-tailed and $P < 0.05$ served as the criterion for statistical significance. All data are presented as ± SD from the mean.

Results

Fast green dye was dissolved in aCSF and microinfused into the NBM of the eight rats that underwent histologic verification of cannula placement and drug spread. It was determined that the drug diffused in a 1-mm radius from the cannula tip. An example is shown in figure 2.[42]

Behavioral data are summarized in figure 3. Rats responding to norepinephrine infusion into the NBM typically showed transient episodes of behavioral arousal characterized by spontaneous and purposeful head and limb movements, as well as sporadic crawling or walking. The rats did not regain their righting reflex for longer than 5 min. The arousal episodes recurred several times over a period of up to 30 min postinfusion. Sham rats that received no infusion remained unresponsive for a duration comparable with the length of infusion experiments. A few rats receiving aCSF infusion showed minor behavioral arousal. The average postinfusion behavioral scores were significantly higher after norepinephrine (2 ± 1) than after aCSF (0.63 ± 0.8) infusion ($P < 0.01$, *t* test).

For further categoric analyses, the rats were classified as responsive or unresponsive, depending on whether they received a behavioral score of 2 or greater, or less than 2, respectively. With these criteria, 8 of the 11 rats infused with norepinephrine were responsive, whereas 8 of the 10 rats infused with aCSF were unresponsive. The proportion of rats

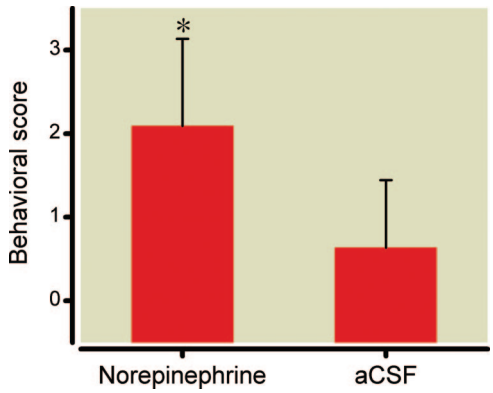

Fig. 3. Behavioral score after pharmacologic modulation of the nucleus basalis of Meynert in all experiments. A significant effect on group mean score was observed with norepinephrine (n = 11) but not with artificial cerebrospinal fluid (n = 10) infusion. Please note that one animal failed to complete both treatment groups because of euthanasia due to removal of the skullcap. *$P < 0.05$ *versus* zero. Error bars ± 1 SD; n = number of rats. aCSF = artificial cerebrospinal fluid.

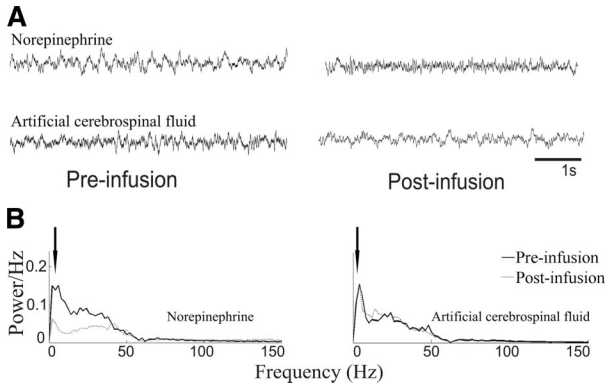

Fig. 4. Electroencephalographic response to norepinephrine and artificial cerebrospinal fluid infusion into the nucleus basalis of Meynert. (*A*) Raw electroencephalogram traces from two rats. In the rat receiving norepinephrine, δ waves are present preinfusion and are reduced postinfusion. In the rat receiving artificial cerebrospinal fluid, δ activity is preserved postinfusion. (*B*) Examples of power spectra from a rat after norepinephrine or artificial cerebrospinal fluid infusion, respectively. Note the decrease in low-frequency power in the rat after norepinephrine infusion, and its preservation in the rat after artificial cerebrospinal fluid infusion (*arrow*).

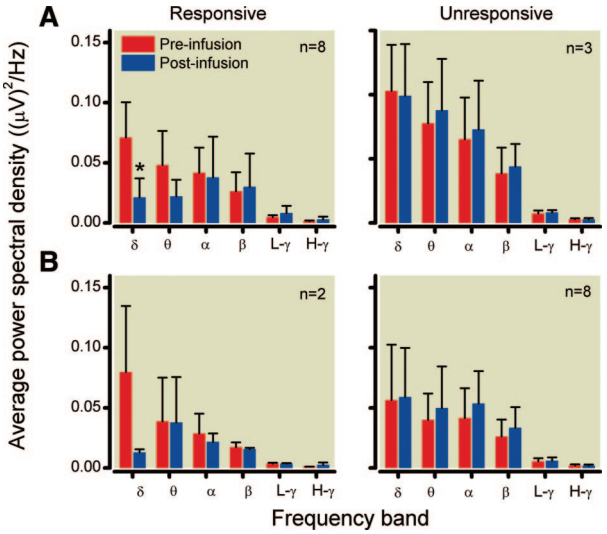

Fig. 5. Electroencephalogram power in various frequency bands before and after pharmacologic manipulation of the nucleus basalis of Meynert in responsive and unresponsive rats. (*A*) Effect of norepinephrine: significant reduction in delta power in most animals (n = 8). (*B*) Effect of artificial cerebrospinal fluid infusion: no change in band powers after artificial cerebrospinal fluid infusion (n = 8). **P* < 0.05 *versus* preinfusion. Error bars are ± 1 SD; n = number of rats.

responding to norepinephrine was significantly higher than those responding to aCSF ($P < 0.0001$, chi-square test).

The electroencephalographic power spectrum was computed for each rat for preinfusion and postinfusion. Example raw electroencephalogram traces are displayed in figure 4A for rats receiving norepinephrine or aCSF infusion, respectively. Slow-wave activity was evident preinfusion in both rats. The rat receiving aCSF continued to display slow-wave activity postinfusion, whereas the electroencephalogram became desynchronized in the rat receiving norepinephrine. The corresponding changes in the power spectra for both rats are displayed in figure 4B.

The effect of NBM microinfusion on electroencephalographic power in five frequency bands was examined. Overall, delta power was significantly reduced after NBM microinfusion (20 ± 14.3%, $P < 0.05$, multivariate ANOVA). None of the other band powers were significantly altered. Moreover, delta power was different as a function of brain region ($P < 0.01$, ANOVA) and drug treatment ($P < 0.05$, ANOVA). Follow-up testing revealed that the reduction in delta power was confined to the frontal cortex (34 ± 13.4%, $P < 0.01$, Student *t* test) with no significant change in the visual cortex (15 ± 13%, $P = 0.97$). Delta power decreased after norepinephrine (47 ± 25%, $P < 0.05$, Student *t* test), but not after aCSF (17 ± 3%, $P = 0.70$) microinfusion.

In figure 5 we compare delta power in responding (behavioral score ≥2) and nonresponding animals from both treatment groups. Again, a significant decrease in delta power was observed in the frontal cortex (73 ± 6%, $P < 0.001$, Student *t* test), but not in the visual cortex (24 ± 10%, $P = 0.84$) of responding animals. The average decrease in frontal cortex delta power was 70 ± 7% in rats that responded after receiving norepinephrine microinfusion. We also found that the presence of behavioral arousal could be significantly predicted by the observed change in frontal cortex delta power ($P < 0.05$, logistic regression).

As an additional measure of electrocortical arousal, cross-approximate entropy was computed preinfusion and postinfusion for each rat. Displayed in figure 6 are individual and group-averaged entropy data after norepinephrine and aCSF treatments. Two-sample Student *t* tests after ANOVA showed that cross-approximate entropy significantly increased from 3.17 ± 0.56 to 3.85 ± 0.29 after norepinephrine ($P < 0.01$), but was unchanged (3.18 ± 0.54 to 3.15 ± 0.53) after aCSF ($P = 0.89$) infusion.

Discussion

We have shown that pharmacologic microinfusion of norepinephrine into the NBM transiently reverses desflurane-induced anesthesia. After microinfusion, electroencephalographic desynchronization was seen as a decrease in δ wave activity while the animals displayed purposeful behavior consisting of limb and head movements and sporadic crawling or walking. The arousal episodes were transient, recurring several times throughout a period of 30 min postinfusion.

Previous studies have implicated a role for the NBM in arousal across natural sleep-wake states: activation of the NBM by microinfusions of norepinephrine, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, *N*-methyl-D-aspartate, or neurotensin[43,44] during sleep produced electroencephalographic activation and waking. Our results confirm and extend this role for the NBM in arousal modulation to

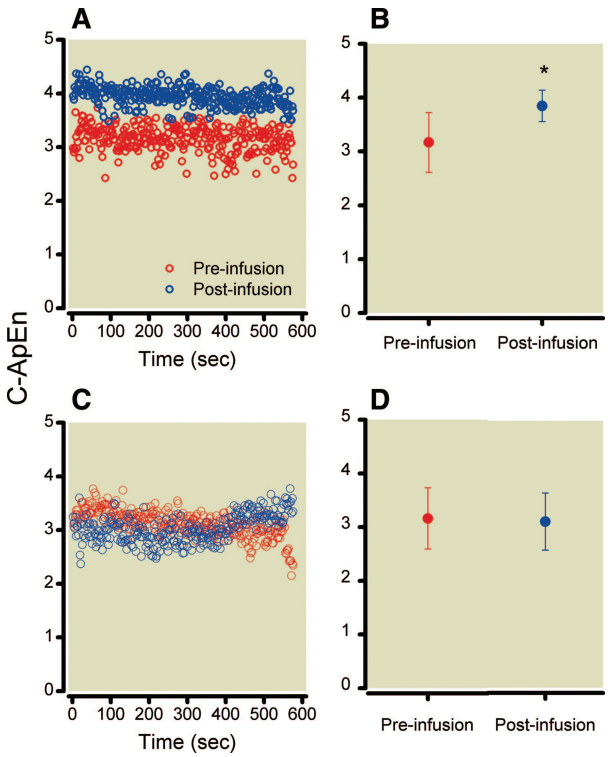

Fig. 6. Cross-approximate entropy (C-ApEn) before and after pharmacologic manipulation of the nucleus basalis of Meynert. (*A*) C-ApEn remained steady preinfusion (*red circles*) and increased after norepinephrine infusion (*blue circles*). (*B*) Box plot of the mean C-ApEn preinfusion and postinfusion with norepinephrine (n = 11). (*C*) C-ApEn remained steady preinfusion (*red circles*) and did not change after artificial cerebrospinal fluid infusion (*blue circles*). (*D*) Box plot of the mean C-ApEn preinfusion and postinfusion with artificial cerebrospinal fluid (n = 10). **P* < 0.01 *versus* zero. Error bars are ± 1 SD; n = number of rats.

general anesthesia, in line with the view that sleep and anesthesia share some common mechanisms and receptor sites of action.[15,35]

Recently, a role for the NBM in the modulation of the state of anesthesia has also been implicated. In particular, the anesthetic potency of propofol was potentiated after lesioning the NBM,[33] and microinjection of histamine into the NBM facilitated electroencephalographic arousal and emergence from isoflurane anesthesia.[34] Nevertheless, to our best knowledge, our results are the first to indicate that pharmacologic manipulation of the NBM is able to modulate the depth of desflurane anesthesia while the agent is administered at a constant hypnotic concentration.

The NBM is a major source of cholinergic innervation to the cerebral cortex.[16] Therefore, it is plausible that the mechanism of behavioral and encephalographic arousal observed after norepinephrine microinfusion was mediated by corticofugal cholinergic modulation. Previous studies demonstrated that the release, presence, and activity of acetylcholine within the cerebral cortex, pontine reticular formation, and striatum are suppressed during general anesthesia.[45–48] Decreased cholinergic transmission lowers the minimum alveolar concentration for isoflurane in the rat,[49] whereas enhanced cholinergic tone in the brain can reverse isoflurane anesthesia.[10] Systemic or cortical application of atropine prevents NBM-mediated electroencephalogram desynchronization.[50,51] Nevertheless, the NBM also sends γ-aminobutyric acid-mediated (GABAergic) input to the cortex[16,52] that may be involved in arousal modulation. In addition, the NBM may not act in isolation, but in concert with other known components of the ascending arousal system (brainstem, hypothalamic, thalamic intralaminar nuclei) that have also been implicated in anesthetic mechanisms.[7,9,35]

In our study, a few animals failed to be aroused by norepinephrine, and most animals failed to be aroused by aCSF. The exact reason why 2 of 10 rats responded to aCSF microinfusion is unknown. A spontaneous change in arousal was unlikely because the sham-operated rats never showed an arousal of any kind. Local excitation from a minor increase in infusion pressure or from the temperature of the infusate cannot be excluded, although precautions were taken to minimize the chance of such effects. In addition, such technical issues should have affected the norepinephrine and aCSF experiments more or less equally, which was not the case.

Volatile anesthetic agents target neurotransmission at multiple brain sites, including the cortex, thalamus, and brainstem,[1,6,15,17,35] and it may therefore be difficult to reverse the volatile anesthetic effect by modulating the NBM alone. Norepinephrine has been shown to depolarize and excite cholinergic cells in the NBM *in vitro*.[53] Cholinergic, GABAergic, and glutamatergic neurons are codistributed within the basal forebrain;[16,54] thus, the consequences of norepinephrine microinfusion into the NBM cannot be easily predicted.

Does the NBM modulate the state of anesthesia? Because we did not measure neural activity in the NBM, we do not know if its activity was altered by desflurane. Previously observed reductions in cortical acetylcholine concentration during anesthesia[45–48] may be due to a direct effect on cholinergic terminals or to an effect on the sources of cholinergic input, including the NBM and brainstem laterodorsal and pedunculopontine tegmental nuclei.[16] As discussed previously, an activation of the NBM by norepinephrine was plausible, and may have reversed the suppressant effect of anesthesia on the NBM or it may have compensated the anesthetic suppression of other arousal pathways. Future studies may test these alternative hypotheses.

Consciousness, Information, and Microarousal

A fundamental but obviously difficult question is whether the behaviorally aroused animals had conscious awareness. Our ability to infer whether the animals regained consciousness during the time they were behaviorally aroused is limited by the lack of objective measures to determine LOC in nonhuman organisms (and strictly speaking, in humans as well). Currently, the LORR is the method of choice for operation-

ally assessing the LOC in rats.[35] However, we cannot infer that the rat was perceptually or volitionally conscious at any point during arousal. For example, one could suggest that their movements may be more akin to a state of sleepwalking. Decerebrate rats can walk and consume food but, by definition, lack conscious awareness.[55] It may be important that in our experiments, electroencephalographic desynchronization after norepinephrine administration was observed in the frontal but not in the visual cortex. Thus, behavioral arousal may have been due to motor cortex activation in the absence of (visual) sensory activation.

It has been suggested that a general attenuation of low-frequency components in the cortical electroencephalogram (δ and θ) indicates the impending return of consciousness after sleep.[43] Alternatively, an increase in the γ band power or coherence may imply the emergence from general anesthesia,[56] although this has been contested by experimental studies.[57–59] In the current study, a significant reduction in delta power in the frontal cortex coincided with behavioral arousal, which is consistent with the known anteriorization of power in general anesthesia and its reversal during emergence.[36,60,61] Because the behavioral scores were predictable by the suppression of delta power, we cautiously surmise that the behavioral response was cortically mediated and, therefore, may have been, at least potentially, volitional and conscious. However, this conclusion will have to be tested with further experimental manipulations of cortical activity.

There is increasing evidence suggesting that anesthetic agents may disrupt functional integration in large-scale cerebral networks.[62,63] Cross-approximate entropy, a statistical parameter, quantifies the statistical dissimilarity between neural signals. In that sense, higher cross-approximate entropy indicates a higher repertoire of independent brain states. The latter has been postulated as one of the requirements for the conscious state as outlined in the information integration theory of consciousness.[64,65] Presumably, impaired or suppressed information processing in the anesthetized state would be revealed by lower cross-approximate entropy values. Information exchange between neural regions is impaired during synchronized states[17,66] as the amount of independent information exchange is reduced. In fact, cross-approximate entropy is decreased under general anesthesia,[41,67] and this can be reversed by cholinergic activation of the cerebral cortex.[10] Our current results are consistent with this observation and suggest that an increase in the repertoire of brain states, and presumably, in the level of consciousness, paralleled the electrocortical activation after norepinephrine infusion into the NBM.

Apparently, the norepinephrine infusion into the NBM was not able to produce permanent or long-lasting reversal of anesthesia. The transient nature of the response may in part be due to the fact that only one of several parallel arousal pathways was modulated while the other pathways remained suppressed by desflurane. Similar spontaneous behavioral and electroencephalographic arousal events were observed during superficial stages of sleep. Schieber *et al.* first described the phenomenon of temporary arousal during natural sleep and termed it 'phases d'activation transitoire'.[68] This terminology was further refined and renamed microarousal.[40] Microarousals increase in frequency from evening to morning, are dynamic, and characterized by decrease in δ waves, increase in higher frequencies, increase in muscle tone, limb and body movement, and increase in heart rate.[69–71] Although the precise roles of microarousals have yet to be established, it has been postulated that they are an integral part of the natural sleep cycle and they serve to ensure the reversibility of sleep.[71] During microarousals, an organism is briefly able to remain connected to the outside environment. This momentary arousal allows for incoming sensory information to be obtained by the central nervous system.[71] A possible clinical significance of this is that if the temporary arousal observed in our study is akin to sleep microarousals then they may, in principle, allow for transient episodes of awareness under anesthesia as well.

In summary, pharmacologic modulation of the NBM by norepinephrine during desflurane anesthesia produced transient behavioral arousals that, when present, were predictable by electroencephalographic desynchronization. Future studies should determine neuronal activity within the NBM preinfusion and postinfusion, measure the induced changes in cortical acetylcholine and GABA release, and test the effect of specific receptor agonists and antagonists to better understand the synaptic events responsible for the observed behavioral and electroencephalographic activation. The interaction of the NBM with other ascending pathways (hypocretinergic, serotonergic, and dopaminergic) that participate in the modulation of arousal should also be investigated.

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