Thalamic Microinjection of Nicotine Reverses Sevoflurane-induced Loss of Righting Reflex in the Rat

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Background: Neuronal nicotinic acetylcholine receptors are both potently inhibited by anesthetics and densely expressed in the thalamus. Brain imaging shows that thalamic activity suppression accompanies anesthetic-induced unconsciousness. Therefore, anesthetic-induced unconsciousness may involve direct antagonism of thalamic nicotinic receptors. The authors test this by separately attempting to block or enhance anesthetic-induced loss of righting in rats using intrathalamic microinjections of nicotine or its antagonist.

Methods: Rats were implanted with a cannula aimed at the thalamus or control locations. A week later, loss of righting was induced using sevoflurane (1.4 ± 0.2%). A dose-parameter study (n = 35) first identified an optimal intrathalamic nicotine dose associated with arousal. Subsequently, this dose was used to pinpoint the thalamic site mediating the arousal response (n = 107). Finally, sevoflurane righting dose and response specificity were assessed after blocking nicotinic channels with intrathalamic mecamylamine pretreatment (n = 8) before nicotine challenge.

Results: Nicotine (150 µg/0.5 µl over 1 min) was the optimal arousal dose, because lower doses (75 µg) were ineffective and higher doses (300 µg) often caused seizures. Nicotine temporarily restored righting and mobility in animals when microinjections involved the central medial thalamus (P < 0.0001, chi-square). Righting occurred despite continued sevoflurane administration. Intrathalamic mecamylamine pretreatment did not lower the sevoflurane dose associated with loss of righting, but prevented the nicotine arousal response.

Conclusions: The reversal of unconsciousness found here with intrathalamic microinfusion of nicotine suggests that suppression of the midline thalamic cholinergic arousal system is part of the mechanism by which anesthetics produce unconsciousness.

◇ This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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MANY authors have proposed that the thalamus and its network interactions with the cerebral cortex plays a critical role in the generation of conscious awareness.[1-5] The thalamus is located in the diencephalon at the center of each cerebral hemisphere and serves as a primary relay station for incoming sensory information. The thalamus also strongly interacts with the motor output centers of the brain.[1] The midline thalamus plays a role in mediating the level of arousal and is considered an extension of the ascending reticular activating system.[3,6,7] Therefore, by inference, anesthetics may cause a loss of consciousness by interrupting or interfering with thalamocortical network interactions.[8] Some support for this idea comes from human neuroimaging studies of various anesthetics, sedative agents, and nonrapid-eye-movement sleep examined at, or near, a loss of consciousness endpoint.[9-18] All of these methods of producing unconsciousness show a regionally specific suppressive effect on relative thalamic activity. This regional interaction between the thalamus and anesthetics has prompted the proposed existence of a localized thalamic consciousness switch,[9] which may be a central component of a broader dose-related anesthetic cascade of effects.[19] The cellular mechanism through which such a switch might work remains unknown.

Two facts converge to suggest that neuronal nicotinic acetylcholine receptors (nAChRs) are a plausible anesthetic target for such a switch mechanism.[1] First, neuronal nAChRs are potently inhibited by many anesthetics at subanesthetic doses.[20,21] This fact suggests that nAChRs are inhibited in significant proportions at the clinically relevant concentration of anesthesia associated with a loss of consciousness.[2] Second, the $\alpha_4\beta_2$ subtype of the nAChR has its highest density of expression in the thalamus,[22] suggesting that the localized decrease in regional thalamic activity seen in anesthesia brain imaging studies might be due to a regionally localized antagonism of nAChRs. Whether the thalamic activity suppression seen with anesthesia is causally related to anesthetic-induced loss of consciousness and whether such activity suppression is causally related to anesthetic-induced antagonism of thalamic nAChRs both remain unanswered questions.

To help identify whether thalamic nicotinic mechanisms play a role in mediating the hypnotic component of inhalational anesthesia, we hypothesized and tested the following ideas. *Experiment 1:* First, if the unconsciousness-producing aspect of anesthesia involves anesthetic-induced antagonism of thalamic nicotinic recep-

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tors, then in a rat given enough sevoflurane to induce a loss of the righting reflex (LORR, a correlate for a loss of consciousness response), an intrathalamic microinfusion dose of nicotine should be found that is capable of restoring the righting reflex, despite continued systemic administration of the anesthetic. *Experiment 2:* Second, if such an arousal response can be found with nicotine, it may be site specific. If so, microinfusions of nicotine outside the key neural circuitry involved in generating arousal will not arouse an anesthetized animal. Therefore, using discrete microinfusions of nicotine into various thalamic structures, it should be possible to pinpoint the thalamic region most associated with mediating arousal. Based on the work of Miller *et al.*,[23] who found that discrete microinfusions of γ-aminobutyric acid (GABA) agonists into the central medial (CM) thalamus would cause a LORR in the rat, we considered the CM thalamus to be our primary target. *Experiment 3:* Third, the thalamus may be the site of an arousal state switching mechanism that serves as both an "on" and an "off" consciousness switch. If so, and if this switch is driven by anesthetic-induced antagonism of nicotinic receptors, the microinfusion of a nicotinic antagonist such as mecamylamine, a potent blocker of the predominate thalamic nicotinic $\alpha_4\beta_2$ receptor subtype, into the thalamus should itself lower or eliminate the dose of sevoflurane needed to cause a LORR. *Experiment 4:* Fourth and finally, a nicotine arousal response should specifically involve the nicotinic receptor. If so, intrathalamic pretreatment with mecamylamine should block the ability of nicotine to generate an arousal response in an anesthetized animal.

Methods and Materials

All research activities were conducted with full approval of the Institutional Animal Care and Use Committee of the University of California, Irvine.

Animals

Sprague-Dawley rats (250–280 g or approximately 9 weeks old on arrival) were obtained from Charles River Laboratories, Inc. (Wilmington, MA). They were housed individually in a temperature-controlled (22°C) colony room, with food and water available *ad libitum*. Animals were maintained on a 12-h light–12-h dark cycle (07:00–19:00 lights on).

Surgery

For all experiments, rats (n = 142) were maintained in the animal colony for 1 week before surgery. Rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal) and given atropine sulfate (0.2 mg, intraperitoneal). Rats were placed into a stereotaxic frame

(Benchmark Digital Stereotaxic; MyNeurolab.com, St. Louis, MO), and a guide cannula (23 gauge) was placed and aimed at the CM thalamus or the various other selected locations. The guide cannula was secured in place with dental acrylic and two small skull screws. All animals were allowed 6–7 days to recover before experiments.

Arousal Response Determinations

The rats were anesthetized with sevoflurane using dose beyond that which caused a LORR (1.4 ± 0.2%). Animals were placed in a rectangular 8-l clear acrylic anesthetizing chamber and exposed to 2.5% sevoflurane in air at 2 l/min until they lost their righting reflex. Anesthetic chamber agent concentrations were monitored continuously during the experiments using a Datex-Ohmeda Ultima Capnomac (Helsinki, Finland) and verified with gas chromatography (model 80123B; SRI Instruments, Redondo Beach, CA). The chamber had a small door on one side through which the animal was initially placed. The chamber also had small ports that served as the anesthetic gas inlet, the microinfusion tubing port inlet, two gas monitor sampling ports, and one gas chromatograph sampling port. When the rat was well anesthetized at the 2.5% sevoflurane level, the door was partially opened, a 25-gauge microinfusion needle was quickly inserted through the guide cannula, and the rat was placed onto its back in the center of the chamber. The needle was attached by a polyethylene tube through the wall of the anesthetizing chamber to a 10-μl syringe (Hamilton, Reno, NV), which was driven by a minipump (Harvard Apparatus, Holliston, MA). The chamber anesthesia concentration was then lowered to 1.2% and verified with gas chromatography. The concentration was held stable for 20 min before a microinfusion was delivered. However, if a rat showed any spontaneous movement during the stabilization period, the chamber concentration was increased by 0.1% increments until the rat remained motionless for at least 20 min. Therefore, the chamber concentration varied slightly depending on a specific animal's behavior, ranging from 1.2 to 1.6%, with a mean and SD of 1.4 ± 0.2%.

Animal arousal responses to microinfusions were categorically graded as one of four different levels. Level 1—no effect—indicated no visible somatic movements or signs of behavioral arousal. Level 2—partial arousal—indicated signs of behavioral arousal, including eye opening, whisker movement, and apparent purposeful movement of head, tail, or feet. Level 3—full arousal—indicated behavioral arousal and included all of the components of level 2 and the additional complete turning of the body onto the stomach, often with ambulation. Level 4—seizures—indicated visible focal or generalized tonic–clonic seizure activity.

Experiment 1: Nicotine Dose Associated with Arousal

Thirty-five animals were used to determine whether a dose of nicotine could be found that would block the LORR to sevoflurane anesthesia. These animals all had cannulae implanted and aimed at the central medial thalamus (coordinates: anteroposterior −3.0 mm from bregma; mediolateral +1.7 mm from midline with 13° tilt; dorsoventral −4.0 mm from skull surface; incisor bar, −3.3 mm from interaural line). It was initially intended that each animal would have five different microinfusions of nicotine at the doses of 0, 75, 100, 150, and 300 μg in 0.5 μl saline vehicle over a 1-min time of microinfusion, at intervals of once a week for 5 weeks. However, it quickly became apparent that the multiple microinfusion design was not feasible and could not be completed because of various problems with the cannula becoming clogged or dislodged after only a few weeks and sometimes after only one microinfusion session. Indeed, 6 animals had only one microinfusion session, 24 animals had two microinfusion sessions, and 4 animals had four microinfusion sessions (1 animal died after surgery). Because the goal of this unblinded parameter study was to find an effective intrathalamic nicotine microinfusion dose that might be associated with an arousal response, the number of intrathalamic microinfusions given varied between the study doses as those found to be ineffective were not emphasized. Therefore, six saline microinfusions, along with five 75-μg, twenty-three 100-μg, twenty-eight 150-μg, and four 300-μg nicotine microinfusions were given. Hence, the dose–response data are best qualitatively interpreted relative to the number of, and type of, behavioral responses found according to the number of successful microinfusions achieved at a particular dose.

Experiment 2: Site Specificity

After determining an intrathalamic nicotine dose capable of demonstrating an arousal response in experiment 1, we then used this probe dose of nicotine in an additional 107 animals to pinpoint the neuroanatomic thalamic region mediating the reversal of sevoflurane-induced LORR. Most animals (n = 61) had their cannulae aimed at the midline anterior CM thalamus. A number of histologic misses were anticipated for this experiment based solely on the relatively small size of the CM thalamus target region, which encompasses approximately 1–2 mm width in the mediolateral dimension, 0.25–0.5 mm depth in the dorsoventral dimension, and 3 mm length in the anteroposterior dimension. A number of animals (n = 30) had their cannulae aimed at a slightly more posterior aspect of the CM thalamus (anterior–posterior −3.8 mm from bregma). Six animals had cannulae specifically aimed at the midline paraventricular thalamic nucleus (coordinates: anteroposterior −3.0 mm, mediolateral +1.2 mm, dorsoventral −4.0 mm),

which has important reciprocal connections with the nucleus accumbens and amygdala. Ten of the 107 animals had cannulae not aimed at midline thalamic structures. Four of these animals had cannulae aimed at the ventral lateral specific thalamic nucleus (coordinates: anteroposterior −3.0 mm, mediolateral +4.0 mm, dorsoventral −4.5 mm), which is a representative component of the specific thalamic system, and the other six of these animals had cannulae implanted into the lateral ventricle as controls for intracerebroventricular injections (coordinates: anteroposterior +8 mm, mediolateral +2.0 mm, dorsoventral −2.5 mm). For all microinfusions, the injection needle extended 2 mm below the end of the guide cannula, except for intracerebroventricular microinfusions, where it only extended 1.5 mm.

Experiment 3: Sevoflurane LORR Dose-sparing Effects of Intrathalamic Nicotinic Antagonist

If intrathalamic nicotine blocks sevoflurane-induced unconsciousness, one might anticipate that the opposite should be true and an intrathalamic nicotinic antagonist should greatly reduce or even eliminate the need for systemic anesthesia to cause a LORR. To test this idea, eight rats from the localization studies that had a positive arousal response to nicotine were selected for further study.

On a different day, these rats underwent a separate determination of the sevoflurane dose that caused a LORR. They were placed into the anesthetizing chamber with a starting chamber concentration of 0.8% sevoflurane as monitored with a Datex-Ohmeda Capnomac and confirmed with gas chromatography. The chamber concentration was then increased by increments of 0.1% every 5 min until the rats did not right themselves when the chamber was physically turned 90° onto its back. When LORR was established, the sevoflurane level was decreased to 0.6%. The rats were allowed to awaken, and a second LORR determination was made. The LORR dose was taken as the average of the two trials that prevented the LORR.

On a different day, another LORR determination was made, but with the addition of an intrathalamic microinfusion of mecamylamine being given to each rat before they entered the anesthesia chamber. Two different doses of mecamylamine were examined in two separate groups of rats (n = 4 per dose). The doses selected, 25 and 50 μg, were given in 0.5 μl vehicle. The doses used approached the limit of the drug's solubility in the respective saline (47 mg/ml, solubility) and ethanol (122 mg/ml, solubility) vehicles.

Experiment 4: Effect of Mecamylamine Pretreatment on Nicotine-induced Arousal

When the animals in experiment 3 demonstrated a LORR response after the intrathalamic mecamylamine microinfusion, they were further anesthetized by in-

creasing the sevoflurane concentration to 2.5% for 10 min. When they were fully anesthetized, the chamber door was partially opened; a different microinfusion needle, filled with nicotine, was quickly inserted into the guide cannula; and the rat was placed onto its back in the center of the chamber. The chamber sevoflurane level was then adjusted to 1.2% and held steady for 20 min. Nicotine (150 μg/0.5 μl over 1 min) was then microinfused into the thalamus, and arousal scores were recorded.

Drugs

Nicotine titartrate HCl and mecamylamine were obtained from Sigma-Aldrich (St. Louis, MO). The drugs were dissolved in buffered saline, pH 7.4–8, except for the one mecamylamine dose that was dissolved in ethanol.

Histology

The rats were killed with an overdose of sodium pentobarbital (250 mg/kg) and subsequently given an intracardiac perfusion of 0.9% saline followed by 10% formalin. Brains were removed from each animal, placed into a 10% formalin solution overnight, and transferred to a 20% sucrose solution for 3–5 days. Brains were sectioned into 50-μm sections using a freezing microtome and stained with thionin. Microinfusion tips were localized blinded to the behavioral data. The microinfusion tip locations are projected onto two representative coronal brain sections taken from the atlas of Paxinos and Watson.[24] However, each microinfusion tip location may, on occasion, be as much as ±0.5 mm away in the anteroposterior dimension from the coronal plane on which it is shown.

Statistics

For experiment 1, the nicotine dose data associated with an arousal response were analyzed qualitatively and are shown as the relative proportions of how each dose delivered generated its subsequent behavioral effects. For experiment 2, the hypothesis that the CM thalamus is a key site involved with mediating the intensity of the arousal response caused by intrathalamic nicotine was examined in two ways using the chi-square technique for categorical data. First, a 4 × 2 contingency table assessed whether any of the four possible behavioral responses (i.e., no effect, partial arousal, full arousal or seizure) that occurred in each rat might be related to the rat's microinfusion involving or not involving the CM thalamus. Second, a more focused 2 × 2 categorical table assessed whether the histology for the full arousal animals involved the CM thalamus at a significantly greater proportion than that expected by chance alone when compared with animals showing no behavioral effect. The data are displayed as the proportion of animals with CM thalamus "hits" or "misses." In addition, an
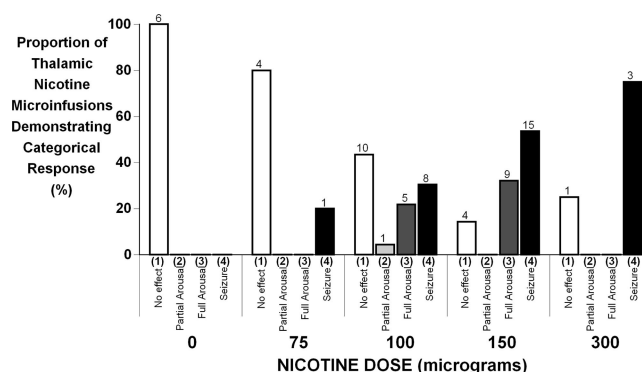

Fig. 1. Intrathalamic nicotine dose parameter study. The data are shown as the proportion of categorical behavioral responses caused by the intrathalamic microinfusion of nicotine at the various escalating doses. The doses of nicotine were microinfused in 0.5 μl saline vehicle over 1 min, and responses were monitored over the next 30 min. Full arousal is seen only with the 100- and 150-μg dose microinfusions, with the 150-μg dose showing a qualitatively higher rate of generating a full arousal response. The numbers above each bar represent the number of infusions given at each respective dose.

intention-to-treat analysis based on intended needle insertion site was also performed for all animals, blinded to the histology findings. For experiment 3, paired t tests were used to assess whether the dose of sevoflurane associated with the LORR differed in the presence or absence of either intrathalamic dose of mecamylamine. The data are displayed as mean and SD. The results for experiment 4, which assessed the ability of intrathalamic mecamylamine to block the arousal effect of nicotine, were assessed qualitatively. For all comparisons, a P value less than 0.05 was considered significant.

Results

Experiment 1: Nicotine Dose Associated with Arousal

The dose-related effects of intrathalamic microinfusions of nicotine on behavioral parameters are shown in figure 1. As shown in figure 1, 100% of the control saline microinfusions did not elicit any observable behavioral reactions. At the other extreme, the 300-μg dose was associated with a 75% seizure rate. Qualitatively, the 150-μg dose of nicotine was most often associated with arousal and was subsequently selected as the site-specific probe dose. However, it was also associated with a 54% seizure rate.

Experiment 2: Site Specificity

As shown in figure 2, the behavioral effects of nicotine (150 μg/0.5 μl over 1 min) microinfusions were determined and localized with histology examination in all 126 animals that received the 150-μg nicotine dose. This number of animals includes the 98 animals that received a single nicotine probe dose plus the 28 animals from experiment 1 that also received the 150-μg nicotine
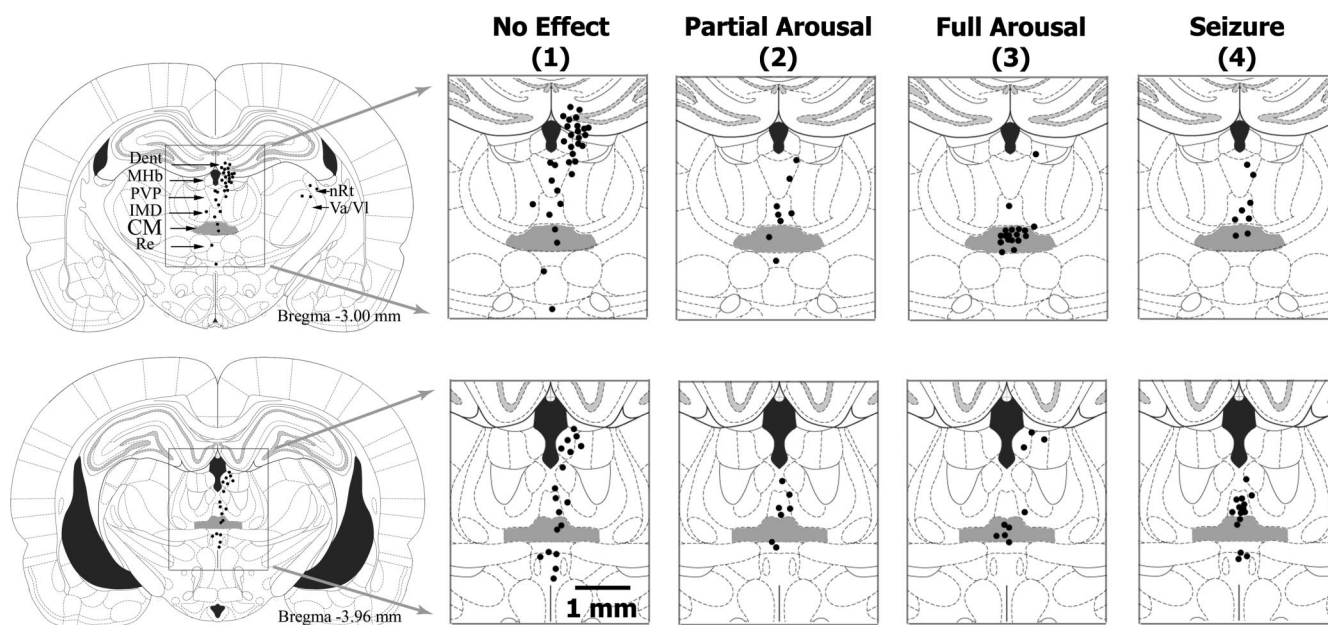
Fig. 2. Histology figures showing locations of microinfusion tips (black dots). The central medial (CM) thalamus is shown as the *highlighted gray shaded area* in the center of each Paxinos and Watson rat atlas figure.[32] Expanded atlas sections are shown as *inserts* to the right and are organized according to the categorical behavioral responses that ensued after the microinfusions of nicotine (150 μg). The data from the no effect group are shown twice, once on the whole atlas slices and once again on the expanded view. The tip locations fall mainly on the anteroposterior coronal planes as shown, but in a few cases they are projected onto the closest atlas figure from up to ±0.5 mm away. Animals showing a full arousal response had infusion tip locations clustering within the CM thalamus. Animals with predominate seizure activity had a clustering of infusion tips within the intermediodorsal (IMD) thalamic nucleus, which is just dorsal to the CM thalamus. CM = central medial thalamus; Dent = dentate gyrus of the hippocampus; IMD = intermediodorsal nucleus; MHb = medial habenular nucleus; nRt = thalamic reticular nucleus; PVP = paraventricular thalamic nucleus (posterior); Re = reuniens thalamic nucleus; Va/Vl = ventral anterior and ventral lateral thalamic nucleus.

dose. Microinfusion needle tip locations are shown relative to each of the four possible behavioral responses ranging from "no effect" to "seizures." The figure shows that a cluster of microinfusion tips are located within the anterior CM thalamus in those animals that demonstrated a full arousal response. Therefore, nicotine demonstrated a site-dependent ability to restore the righting reflex and mobility in those animals whose microinfusion sites were within approximately a 1-mm target zone centered on the anterior (anteroposterior -3.0 mm from bregma) CM thalamus. Righting occurred despite the continued systemic presence of the anesthetic. Righting occurred on average ($\pm$ SD) 301 $\pm$ 159 s after the end of the nicotine microinfusion. The wakefulness restoring effect was only temporary and lasted a median time of 60 s (interquartile range, 43–156 s), as timed from the restoration of righting to the point where the animals were again immobilized.

The histology data suggested that the behavioral effects could be interpreted relative to whether the CM thalamus was involved in generating a particular behavioral effect. This result is shown in figure 3. The figure shows the proportional breakdown of the four possible behavioral responses according to whether the microinfusions "hit" or "missed" the CM thalamus for those microinfusions that targeted the midline thalamus (*i.e.*, excluding the four animals that targeted the ventral anterior and ventral lateral thalamus and the six intracere-

broventricular injections). A 4×2 contingency table testing the null hypothesis that the four possible behavioral responses were unrelated to the placements of the microinfusion tips either involving or not involving the CM thalamus showed a significant effect ($P < 0.0001$, chi-square). Therefore, rejecting the null hypothesis of
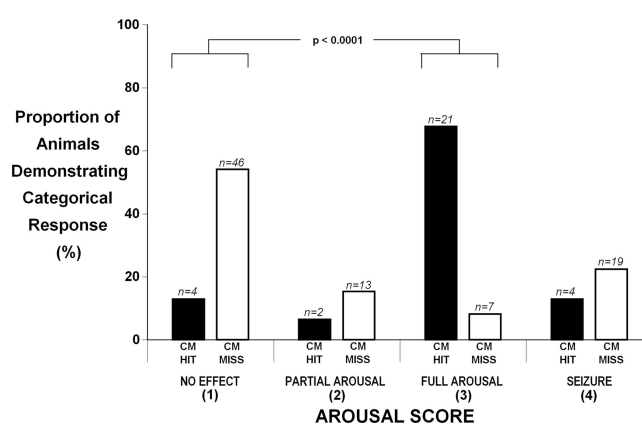

Fig. 3. Percentage of arousal scores categorized by whether the histology findings involved the central medial (CM) thalamus. Those animals with microinfusion cannula tips located within the CM thalamus had a significantly greater chance ($P < 0.0001$) of demonstrating an arousal response (67% of the animals with CM hits) to intrathalamic microinfusion of nicotine (150 μg) and a temporary reversal of the unconsciousness component of anesthesia than did those animals where the microinfusions missed the CM thalamus. *Dark bars* = CM thalamic hits; *light bars* = CM thalamus misses. The number of animals demonstrating each respective response is shown above each bar.
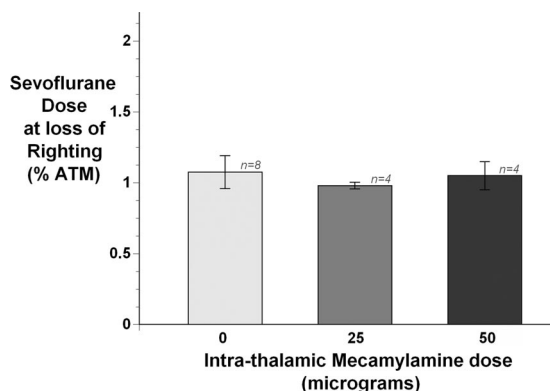
Fig. 4. The figure shows the dose of sevoflurane that causes a loss of the righting reflex in animals when they are given various intrathalamic doses of the nicotinic antagonist, mecamylamine before the determination. All animals had baseline loss of the righting reflex determinations made on a different day, and then four animals received the 25-μg dose of mecamylamine (in 0.5 μl saline) and four animals received the 50-μg dose in 0.5 μl ethanol. No significant effect on the dose of sevoflurane causing the loss of the righting reflex is apparent with either mecamylamine dose. Data are mean and SD.

no effect and suggesting the site of infusion was important for causing an arousal response. A 2 × 2 contingency table further testing the null hypothesis that "full arousal" *versus* "no effect" was unrelated to microinfusion tip placement either involving or not involving the CM thalamus was also significant ($P < 0.0001$, chi-square).

Nicotine microinfusions that clustered in other brain areas, including the ventrolateral nucleus (n = 2), reticular thalamus (n = 2), paraventricular thalamus (n = 14), or hippocampus (n = 11), did not produce an arousal effect, but the numbers of injections made in some of these areas are inadequate to exclude an effect. The directed intracerebroventricular (n = 6) microinfusions given into the lateral ventricle were also without effect. Among the 142 rats whose data were used for the intention-to-treat analysis, arousal was obtained for 21 of 126 in the treatment group targeting the CM thalamus and for none of the 16 rats that were non-CM thalamus targeted controls. This was a statistically significant difference ($P < 0.001$).

A 2 × 2 contingency table testing the null hypothesis that the microinfusions causing or not causing seizures were unrelated to microinfusion tip locations involving or not involving the intramedullary thalamic nucleus was significant ($P < 0.001$, chi-square). Therefore, rejecting the null hypothesis of no effect and suggesting the site of infusion was important for causing a seizure response.

Experiment 3: Sevoflurane LORR Dose-sparing Effects of Intrathalamic Nicotinic Antagonist

The effect of intrathalamic microinfusions of the nicotinic antagonist mecamylamine on the dose of sevoflurane causing a LORR in animals is shown in figure 4. Neither the 25-μg intrathalamic mecamylamine dose (P

= 0.22) nor the 50-μg mecamylamine dose ($P = 1$) significantly reduced the amount of sevoflurane required to induce a LORR.

Experiment 4: Effect of Mecamylamine Pretreatment on Nicotine-induced Arousal

None of the eight animals showed any signs of behavioral arousal with the intrathalamic nicotine challenge attempt that followed the mecamylamine pretreatment.

Discussion

To summarize, there were five primary findings of this study. First, in a parameter study, an intrathalamic microinfusion dose of nicotine was found that could block the LORR response caused by sevoflurane anesthesia and awaken an anesthetized animal while it was still in the presence of a dose of sevoflurane that should keep it unconscious. Second, the neuroanatomic site mediating this arousal response was identified as the central medial thalamic nucleus. Microinfusions missing this site, intracerebroventricular microinfusions, or microinfusions in other brain areas such at the hippocampus, ventral lateral thalamus, reticular thalamic nucleus, or posterior paraventricular thalamic nucleus, were less likely to produce arousal. Third, the neuroanatomic site mediating a seizure response to intrathalamic microinfusion of nicotine is potentially identified as the intermedial dorsal thalamic nucleus. However, the intermedial dorsal thalamus is immediately adjacent to the central medial thalamus. Therefore, given the diffusion characteristics of the microinfusion technique used, the actual site of seizure induction is still in all likelihood the central medial thalamus. Fourth, an intrathalamic microinfusion of mecamylamine, a nicotinic antagonist, did not change by itself the dose of sevoflurane needed to induce the LORR. If the CM thalamus were the "on/off" switch for anesthetic-induced unconsciousness, one would have expected that the LORR dose of sevoflurane would have changed substantially with intrathalamic mecamylamine pretreatment. The fact that it did not suggests that the CM thalamus may represent more specifically only the "on," and not the "off," component of an arousal state switching mechanism. Fifth and finally, an intrathalamic microinfusion of mecamylamine did prevent a subsequent intrathalamic microinfusion arousal dose of nicotine from causing an arousal response. This strongly suggests that nicotinic acetylcholine receptors play a part in regulating the "on" component of the switching mechanism.

The arousal properties of cholinergic agonists have long been recognized.[25] Nicotine is known to arouse sleeping animals and to desynchronize the electroencephalogram in anesthetized animals.[26] Systemic administration of the cholinesterase inhibitor physostigmine

reversed propofol-induced unconsciousness in 9 of 11 human subjects during a continuous microinfusion of propofol.[27] The reversal effect was blocked by scopolamine pretreatment, suggesting the involvement of muscarinic receptors in the arousal response. Physostigmine also has some potential to reverse the unconsciousness of inhalational anesthesia, but the reversal response is less reliable, occurring in only five of eight test subjects given sevoflurane anesthesia.[28] Nonetheless, when taken together, human observation studies strongly imply that anesthetic-induced suppression of central cholinergic activity plays a causative role in mediating the unconsciousness component of anesthesia.

Further support for a cholinergic hypothesis of anesthetic-induced unconsciousness comes from animal work in which intracerebroventricular microinfusions of the muscarinic agonist oxotremorine were given to rats during 1.0% isoflurane inhalation and measurements were made of the cross-approximate entropy of the bihemispheric frontal electroencephalogram.[29] Oxotremorine microinfusions reversed the electroencephalographic depressant effects of the anesthesia and allowed animals to make spontaneous motor movements suggestive of a return to consciousness, although a full restoration of the righting reflex could not be confirmed with the experimental apparatus used.

However, not all studies support a role for suppression of cholinergic mechanisms in mediating the effects of anesthesia. Systemic administrations of nicotinic antagonists do not induce anesthesia or have anesthetic-sparing effects.[30,31] Genetically altered knockout mice lacking a β_2 nicotinic receptor (the predominate one in the CM thalamus) do not show any measurable changes in anesthetic dose requirements when compared with normal mice.[31] In addition, *in vitro* work does not confirm a role for nicotinic antagonism in mediating spinal mechanisms of anesthetic effects.[32] In one sense, these findings are consistent with our observation that high doses of mecamylamine delivered directly to the CM thalamus did not change the dose at which the LORR occurred with sevoflurane. This suggests that nicotinic antagonism in the midline thalamus is not, by itself, sufficient to generate a LORR response.

Why intrathalamic mecamylamine did not reduce the amount of anesthesia needed to cause unconsciousness is not clear. The nicotine binding site and the antagonist binding site occur on different subregions of the nicotinic receptor,[33,34] so perhaps anesthetics hinder the antagonist from reaching its site of action and thus prevent any additive antagonistic effect on the nicotinic receptor. Another possibility might involve localized neurocircuitry. Nicotinic receptors tend to be located presynaptically on GABA neurons.[35] The work of Miller *et al.*[23] shows that GABAergic responses in the CM thalamus are involved in regulating arousal and seizures. But how GABAergic mechanisms in the CM thalamus

interact with nicotinic mechanisms and how both are affected by anesthetics of various types remain to be elucidated. Yet another possibility is that the CM thalamus is not the site of an anesthetic-mediated consciousness "off" switch. Anesthetics may block arousal by inhibiting these thalamic cells (and other systems not specifically tested), but that does not mean anesthetics must also cause unconsciousness by acting at this one single site.

One potential site for an anesthetic-induced consciousness "off" switch comes from the work of Devour and Zalkind.[36] These investigators identified a brainstem region where localized injections of anesthetics rapidly caused atonia and apparent unconsciousness in rats. Another possibility for an "off" switch site comes from the work of Ma *et al.*,[37,38] who discovered that the septal–hippocampal system mediates a portion of the sedative component of halothane and pentobarbital anesthesia. Still, there does not need to be a singular switch site through which anesthetics might work to cause unconsciousness. Indeed, the more global suppressive actions of anesthetics might represent a functional neural correlate of an "off" switch, and some evidence suggests that an "off" switch is more likely to be related to the ability of anesthetics to enhance inhibition in a widespread manner. In support of a GABAergic mechanism, anesthetics do inhibit cortical neuronal functioning in animals *in vivo* at doses causing loss of consciousness that closely approximate the *in vitro* doses required to affect GABA receptors.[39] Furthermore, propofol inhibits human regional cerebral glucose metabolism in a pattern that correlates with the known regional distribution of GABA receptors.[40] Both observations suggest that anesthetic-induced unconsciousness may be due to a generalized depression of function throughout the brain, rather than through any specific interaction with a switch-like mechanism.

Nevertheless, a case has been made for the sedative component of anesthesia being due to interactions with specific components of normal sleep pathways.[41,42] Nelson *et al.*[43] identified that the hypothalamus plays a role in mediating the sedative component of anesthesia for GABAergic agents. Interestingly, the hypothalamic circuitry identified by that work strongly interacts with the arousal circuitry of the CM thalamus.[44]

In this study, the microinfusion technique of delivering nicotine directly to a discrete brain region allowed for the delivery of a greater dose of nicotine than is possible with systemic administration. The typical dose of nicotine used in pain studies and one anesthesia related study is in the range of 0.8–1.0 mg/kg subcutaneously.[31,45,46] Such a dose leads to peak levels of nicotine in the rat brain in the range of 2 nMol/g brain tissue that peak within 5 min and have a brain tissue half-life of around 53 min.[46] The 150 μg in 0.5 μl nicotine dose used in this study equates to a direct regional brain dose of approx-

imately 3,200 nMol/g brain tissue, or roughly 1,600 times the dose typically used in systemic studies. The unblinded nature of our parameter study, coupled with its subjective endpoint, may have biased our selection of the nicotine probe dose. A more controlled study may have found a more optimal dose. In any event, systemic studies would be unable to deliver such regionally high brain concentrations of nicotine because the rat LD_{50} for systemic nicotine is 50 mg/kg.

The LORR determinations used in experiment 3 used a rapid increase in sevoflurane dose technique that is expected to overestimate the dose causing a LORR. This rapid determination technique was needed to ensure that the intrathalamic mecamylamine would still be pharmacologically active at the proper time when the LORR point was reached in experiment 3 and when the nicotine reversal attempt was made in experiment 4.

Current theories of anesthesia suggest the anesthetic state occurs through multiple mechanisms at multiple sites throughout the central nervous system.[47–50] The current findings establish that the CM thalamus is a component of an arousal state switching mechanism influenced by anesthesia.[9] Clinical studies support a fundamental role for the intralaminar thalamus in mediating various aspects of human arousal. Evidence suggests that CM-parafascicular thalamic neurons play a role in selective human attention.[51] A study of awakening showed that relative cerebral blood flow first returns to the medial thalamus and reticular formation when a person awakens from stage 2 sleep.[52] Further support that the CM thalamus is involved with mediating seizures and arousal comes from intrathalamic stimulation studies. CM stimulation has efficacy for controlling or even eliminating seizures in select patients.[53] Furthermore, CM stimulation has potential for restoring consciousness to those in a vegetative state.[54]

We postulate that the CM thalamus is able to control levels of arousal, attention, and seizures by controlling the amount of corticothalamic reentrant neural activity.[55] By this view, too little reentry results in unconsciousness, whereas too much reentry causes seizures to predominate. This reentry gating hypothesis fits with the strategic neuroanatomy of the CM thalamus and its primary widespread cholinergic and orexinergic modulated influences on the cortex.[56–58]

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