

# Basal Forebrain Histaminergic Transmission Modulates Electroencephalographic Activity and Emergence from Isoflurane Anesthesia

Tao Luo, M.D., Ph.D.,\* L. Stan Leung, Ph.D.†

**Background:** The tuberomammillary histaminergic neurons are involved in the sedative component of anesthetic action. The nucleus basalis magnocellularis (NBM) in the basal forebrain receives dense excitatory innervation from the tuberomammillary nucleus and is recognized as an important site of sleep-wake regulation. This study investigated whether NBM administration of histaminergic drugs may modulate arousal/emergence from isoflurane anesthesia.

**Methods:** Microinjections of histaminergic agonists and antagonists were made into the NBM of rats anesthetized with isoflurane. The changes in electroencephalographic activity, including electroencephalographic burst suppression ratio and power spectra, as well as respiratory rate, were recorded under basal conditions and after NBM injection. Time to resumption of righting reflex was recorded as a measure of emergence from anesthesia.

**Results:** The rats displayed a burst suppression electroencephalographic pattern at inhaled isoflurane concentrations of 1.4–2.1%. Application of histamine (1  $\mu\text{g}/0.5 \mu\text{l}$ ) to the NBM reversed the electroencephalographic depressant effect of isoflurane; *i.e.*, electroencephalographic activity shifted from the burst suppression pattern toward delta activity at 1.4% isoflurane, and the burst suppression ratio decreased at 2.1% isoflurane. Histamine-evoked activation of electroencephalography was blocked by NBM pretreatment with a H1 receptor antagonist, triprolidine (5  $\mu\text{g}/1 \mu\text{l}$ ), but not by a H2 receptor antagonist, cimetidine (25  $\mu\text{g}/1 \mu\text{l}$ ). The respiratory rate was significantly increased after histamine injection. NBM application of histamine facilitated, while triprolidine delayed, emergence from isoflurane anesthesia.

**Conclusions:** Histamine activation of H1 receptors in the NBM induces electroencephalographic arousal and facilitates emergence from isoflurane anesthesia. The basal forebrain histaminergic pathway appears to play a role in modulating arousal/emergence from anesthesia.

NEURONAL histamine in the central nervous system plays a crucial role in the regulation of normal sleep-

wake cycles.<sup>1</sup> Histamine neurons in the brain are located predominantly in the tuberomammillary nucleus (TMN) of the posterior hypothalamus.<sup>2</sup> Increased activity of the histaminergic neurons has been implicated in the facilitation of behavioral wakefulness.<sup>3,4</sup> Histamine levels in the cortex are highest in active waking, lower in slow-wave sleep, and lowest during rapid eye movement sleep.<sup>5</sup> Histamine acts on G-protein coupled receptors in the brain, with H1 and H2 receptors mainly exciting the postsynaptic membrane and H3 receptors suppressing the presynaptic release of histamine and other neurotransmitters.<sup>6</sup>

TMN neurons receive a strong  $\gamma$ -aminobutyric acid-mediated input, which is responsible for quieting them during sleep.<sup>7</sup> Both *in vivo* and *in vitro* studies have shown that the sedative action of some general anesthetics (pentobarbital and propofol) is mediated by  $\gamma$ -aminobutyric acid receptor type A on TMN histaminergic neurons.<sup>8,9</sup> The  $\gamma$ -aminobutyric acid-mediated anesthetic agents that act on  $\gamma$ -aminobutyric acid receptor type A may decrease TMN neuronal firing and histamine release, thus reducing histamine's excitation of the cortical activation circuitry. If suppression of histamine release promotes sedation, it may be hypothesized that promoting histaminergic activity will decrease the depth of general anesthesia and facilitate emergence from anesthesia.

The basal forebrain is highly interconnected with a number of brain regions involved in sleep-wake regulation, including the noradrenergic neural networks of the locus coeruleus, serotonergic system of the dorsal raphe nucleus, as well as the TMN region.<sup>10-12</sup> Extensive evidence indicates that the basal forebrain receives dense excitatory innervation from the TMN.<sup>13,14</sup> The nucleus basalis magnocellularis (NBM) of the basal forebrain is important in the regulation of neocortical electrical activity,<sup>15,16</sup> and histamine has been reported to modulate the activity of NBM neurons in both *in vivo* and *in vitro* studies.<sup>17,18</sup> Our laboratory has previously shown that selective inactivation of the basal forebrain structures potentiated the sedative effect of both intravenous and inhaled anesthetics.<sup>19</sup> A study from Laalou *et al.* further demonstrated that the anesthetic potency of propofol was increased in rats with a basal forebrain lesion.<sup>20</sup> While these studies suggest an important role of the basal forebrain in mediating general anesthesia, the participation of histaminergic transmission in the basal forebrain has not been established.

The aim of the present study was to investigate the effect of altering basal forebrain histaminergic transmis-

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\* Postdoctoral Fellow, Department of Physiology and Pharmacology, † Professor, Department of Physiology and Pharmacology and Program in Neuroscience, The University of Western Ontario.

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Address correspondence to Dr. Leung, Department of Physiology and Pharmacology, Medical Sciences Building, The University of Western Ontario, London, Ontario N6A 5C1, Canada. sleung@uwo.ca. Information on purchasing reprints may be found at [www.anesthesiology.org](http://www.anesthesiology.org) or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

sion on arousal/emergence from isoflurane anesthesia. First, we evaluated the changes in electroencephalographic activity caused by administration of histamine into the NBM of isoflurane-anesthetized rats. Second, we investigated the histamine receptor subtype responsible for electroencephalographic activation. Third, we examined the influence of NBM administration of histaminergic drugs on the emergence time from isoflurane anesthesia.

## Materials and Methods

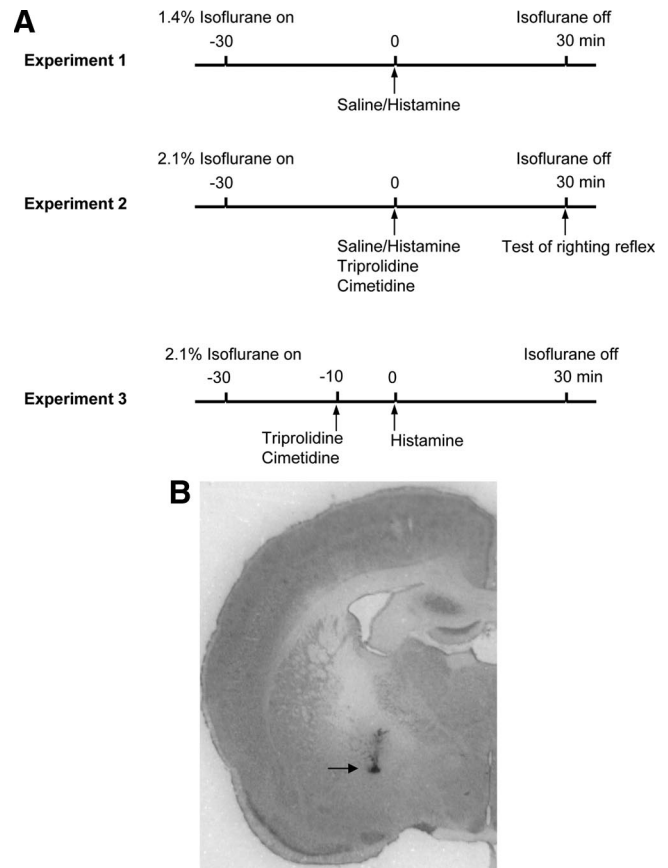
### Animals and Surgery

The experimental procedures and protocols used in this investigation were approved by the Animal Use Committee at the University of Western Ontario (London, Ontario, Canada). Male Long Evans rats weighing 240–280 g were housed under constant temperature ( $23 \pm 1^\circ\text{C}$ ) and a 12 h light, 12 h dark cycle (light period starting at 07:00 h) with *ad libitum* access to food and water.

The rats were deeply anesthetized under sodium pentobarbital (60 mg/kg intraperitoneal) and placed in a stereotaxic frame. All coordinates were relative to the bregma, with the bregma and lambda on a horizontal plane according to the rat brain atlas of Paxinos and Watson.<sup>21</sup> Electroencephalographic electrodes were placed in the left frontal cortex (A 3.7, L 3.2, and 1.7 mm deep [D]) and dorsal hippocampus (P –3.8, L  $\pm 2.5$ , D 3.3) from the skull surface. The electrodes were 125- $\mu\text{m}$  stainless-steel wires that were Teflon-insulated except at the cut tips. A screw in the skull over the cerebellum served as both the recording reference and the recording ground. Stainless steel guide cannulae (23-gauge) were implanted bilaterally for subsequent infusions into the NBM (A –1.4, L  $\pm 2.5$ , D 8.5). The cannulae and electrodes were secured firmly to the skull with dental acrylic. The rats were allowed to recover from surgery for at least 7 days before experimentation.

### Experimental Procedures

All experiments were conducted between 9 and 19 h. The experimental design of this study is illustrated in figure 1A. On the day of the experiment, anesthesia was initially induced in a chamber using 4–5% isoflurane (vaporizer setting) in 100% oxygen (2 l/min). After loss of the righting reflex (LORR) and evaluation of changes in respiratory rate, the animals were exposed to 1, 1.4, or 2.1% isoflurane (with 100% oxygen, flow rate 1 l/min) *via* a facemask connected to a scavenging system. The isoflurane vaporizer was used throughout the study, and stability of output over time was verified by an infrared gas analyzer. The 1%, 1.4%, and 2.1% isoflurane were considered to be the minimum alveolar concentration values of 0.7, 1.0, and 1.5, respectively, in rats.<sup>22</sup> Body temperature was determined rectally and maintained at



**Fig. 1.** Experimental procedures and histologic section of the injection cannula. (A) All animals were subjected to 60 min inhalation of either 1.4% or 2.1% isoflurane. The changes in neocortical electrical activity and behavioral arousal after histaminergic agonist/antagonist manipulations were studied (see experimental procedures for details). (B) Representative photomicrograph of a thionin-stained coronal section (60  $\mu\text{m}$  thick) through the basal forebrain, showing the track of the injection cannula. The arrows point to the injection sites in the nucleus basalis magnocellularis.

$37.0$ – $37.5^\circ\text{C}$  with a heating lamp. In Experiment 1, a 30-gauge injection cannula was introduced through the guide cannula into the NBM after a 30-min equilibration period with 1.4% isoflurane. The injection cannula was connected to a 20- $\mu\text{l}$  Hamilton syringe by means of polyethylene10 tubing, and saline or histamine (1  $\mu\text{g}/0.5$   $\mu\text{l}$ ) was bilaterally infused into the NBM over 5 min. This was followed by another 25 min of isoflurane administration to give a total isoflurane administration time of 60 min in which electroencephalography and behavior were studied. In Experiment 2, all rats were subjected to 2.1% isoflurane anesthesia for 60 min, and received an NBM injection of saline, histamine (1  $\mu\text{g}/0.5$   $\mu\text{l}$ ), selective H1 receptor antagonist tripolidine (5  $\mu\text{g}/1$   $\mu\text{l}$ ), or selective H2 receptor antagonist cimetidine (25  $\mu\text{g}/1$   $\mu\text{l}$ ) alone 30 min before discontinuing isoflurane. In Experiment 3, all rats were subjected to 2.1% isoflurane anesthesia for 60 min, and received an NBM injection of histamine 30 min before discontinuing isoflurane; with the addition of an NBM injection of tripolidine or cime-

tidine 10 min before histamine administration. Each rat was subjected to drug/vehicle application, in random order, separated by at least 4 days. In a separate experiment, the effect of isoflurane on cortical electroencephalographic activity was evaluated. Stable electroencephalographic signals were recorded in rats during awake immobility, and > 15 min after inhaling 1–2.1% isoflurane.

#### *Electroencephalography Recording and Analyses*

Electroencephalography at a depth electrode was recorded using the screw above the cerebellum as the reference. Electroencephalographic signals were amplified by Grass P511 amplifiers, recorded on paper, and digitized by a 12-bit analog to digital converter at 1 kilohertz. The high-pass filter was set at 0.3 hertz (Hz) (0.3-decibel drop-off point) on the Grass P511 amplifier. Averaging 5 consecutive points sampled at 1 kilohertz effectively reduced the sampling rate to 200 Hz and added a low-pass digital filter (0.3-decibel drop-off point) at 84 Hz. Electroencephalography was recorded every minute, starting immediately after the onset of LORR until the discontinuation of isoflurane. Every minute of digitized electroencephalography was manually reviewed to exclude segments with artifacts, and at least six segments of electroencephalography (> 30 s), each segment of 5.12 s and 1,024 points sampled at 200 Hz, were used for power spectral analysis.<sup>23</sup>

When isoflurane concentration was maintained at 1.4% or 2.1%, the cortical electroencephalography exhibited a burst suppression pattern; *i.e.*, an electroencephalographic pattern where high-amplitude bursts are interrupted by low-amplitude suppressions. The burst suppression ratio (BSR) was calculated using previously established methods.<sup>24,25</sup> Briefly, electroencephalography suppression was defined as amplitude less than a preset threshold value, and an electroencephalographic burst was terminated when the electroencephalographic amplitude returned to values below the threshold for 100 ms. The BSR was calculated as the percentage of electroencephalographic suppression in a 60-s interval. A BSR of 100% indicates electroencephalographic silence. The threshold values were manually estimated for each of the rats individually and were three SEM of the clearly nonbursting electroencephalography segments. The threshold values were chosen so that visual inspection confirmed that all or almost all periods with electroencephalographic silence were included as periods of electroencephalographic suppression.

#### *Behavioral Arousal*

Behavior was monitored continuously during the 60 min of anesthesia. The respiratory rate during anesthesia was measured as an index of the depth of anesthesia.<sup>26</sup> In Experiment 2, after discontinuation of isoflurane, the animals were placed in the supine position,

and the time to regain the righting reflex after the anesthetic vaporizer was shut off was recorded as the emergence time.

#### *Drugs*

The following chemicals were purchased from Sigma-Aldrich Co. (Oakville, Ontario, Canada): histamine (H7125), cimetidine (C4522), and triprolidine (T6764). Cimetidine was dissolved in 0.15 M hydrochloric acid with saline and then adjusted to neutral pH with 1 M sodium hydroxide. All other drugs were dissolved in 0.9% physiologic saline solution and administered intracerebrally in a volume of 0.5–1.0  $\mu$ l. The histamine doses chosen for this study were based on previous *in vivo* experiments. Histamine at doses of 5–60  $\mu$ g/ $\mu$ l in the hypothalamus,<sup>27</sup> and 1–200  $\mu$ g in the nucleus accumbens were found to induce behavior arousal.<sup>28</sup> The doses of triprolidine and cimetidine were chosen based on their ability to fully block the histamine responses *in vitro*,<sup>29,30</sup> and confirmed by our own preliminary results *in vivo*. In studies based on intracerebral infusion of muscimol,<sup>23,31</sup> a  $\gamma$ -aminobutyric acid receptor type A agonist with a molecular weight in the same order of magnitude as histamine, triprolidine, and cimetidine, was found to spread < 1 mm outside of the targeted area. Thus, a histaminergic drug is expected to exert an effect within 1 mm of the intended target in the NBM.

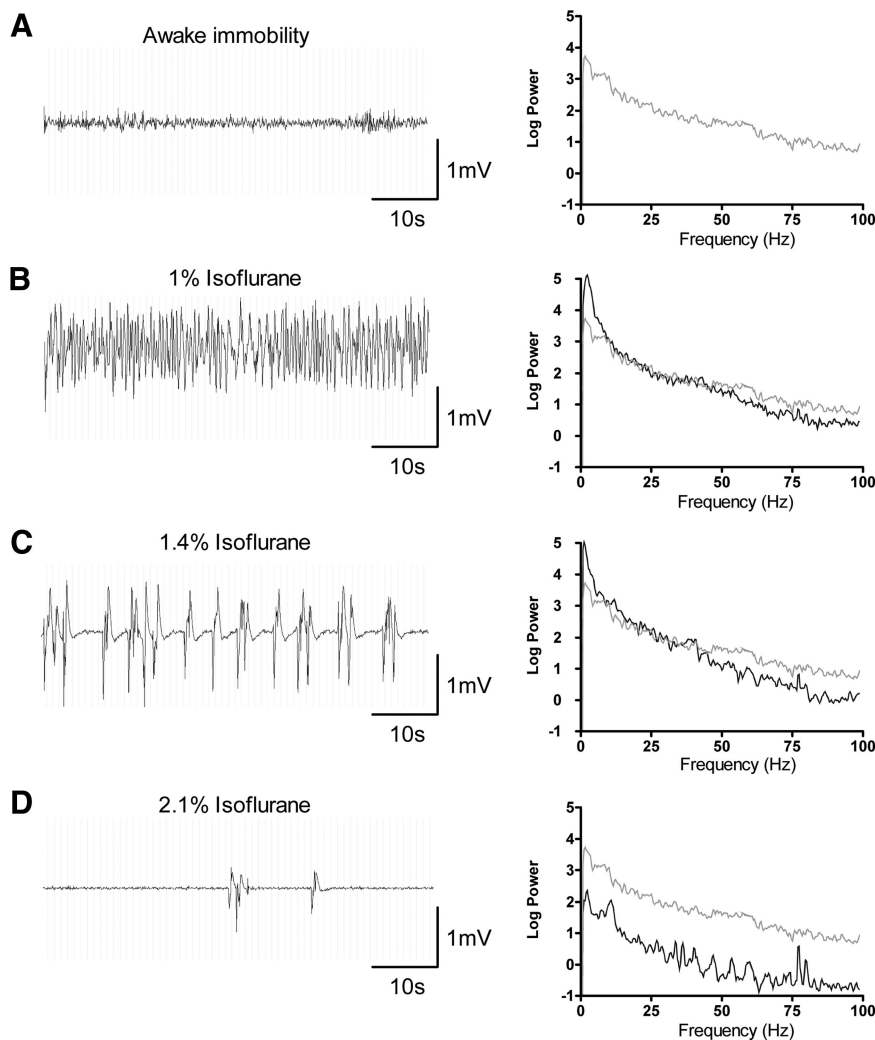
#### *Histology*

On completion of the experiments, all rats were killed with an overdose of urethane and then perfused intracardially with 0.9% saline followed by 10% formalin. The electrodes and the sites of cannula infusion were verified in 60- $\mu$ m frozen sections of the brain stained with thionin. Only data from rats with cannulae confirmed at the intended sites were used for analyses. A representative histologic micrograph showing the location of the ventral tip of the microinjection cannula in the NBM is shown in figure 1B.

#### *Statistical Analysis*

Data were expressed as mean  $\pm$  SEM. GraphPad Prism software version 4.0 (GraphPad Prism, Inc., San Diego, CA) was used for the statistical evaluation. The effect of histamine on electroencephalographic power was evaluated by paired *t* test. Within-group analysis for electroencephalographic BSR were conducted by one-way ANOVA, with time as the repeated measure to determine whether a treatment had a significant effect within each experimental group, and between-group comparisons for electroencephalographic BSR were compared using two-way ANOVA (drug  $\times$  time) with repeated measures on one variable (time). *Post hoc* between-group comparisons of specific samples were performed using *t* tests with Bonferroni corrections. The effects of histaminergic agent on respiratory rate and emergence from isoflu-





**Fig. 2.** Representative examples of electroencephalographic activity and corresponding power spectra recorded in the frontal cortex during (A) awake immobility, and > 15 min after inhaling (B) 1%, (C) 1.4%, and (D) 2.1% isoflurane in 100% oxygen (1 l/min). As compared with the low-voltage fast activity during awake immobility (A), 1% isoflurane increased slow waves in the electroencephalography, which are shown to be < 20 hertz and with a delta peak ( $\delta$ ) in the power spectrum (B). 1.4% isoflurane induced a burst suppression pattern (C), and 2.1% isoflurane increased the periods of isoelectric electroencephalography, resulting in a large reduction in electroencephalographic power at all frequencies (D). Calibration of 1 mV and 10 s apply to all electroencephalographic traces. The power spectrum in (B–D) (black dark trace) is overlaid on the awake immobility spectrum (light gray trace). The power spectra were plotted in logarithmic units, with a calibration of 4.27 log units = 0.5 mV peak-to-peak sine wave.

ane anesthesia were evaluated by one-way ANOVA, followed by *post hoc* analysis (Bonferroni).  $P < 0.05$  was considered to be statistically significant.

## Results

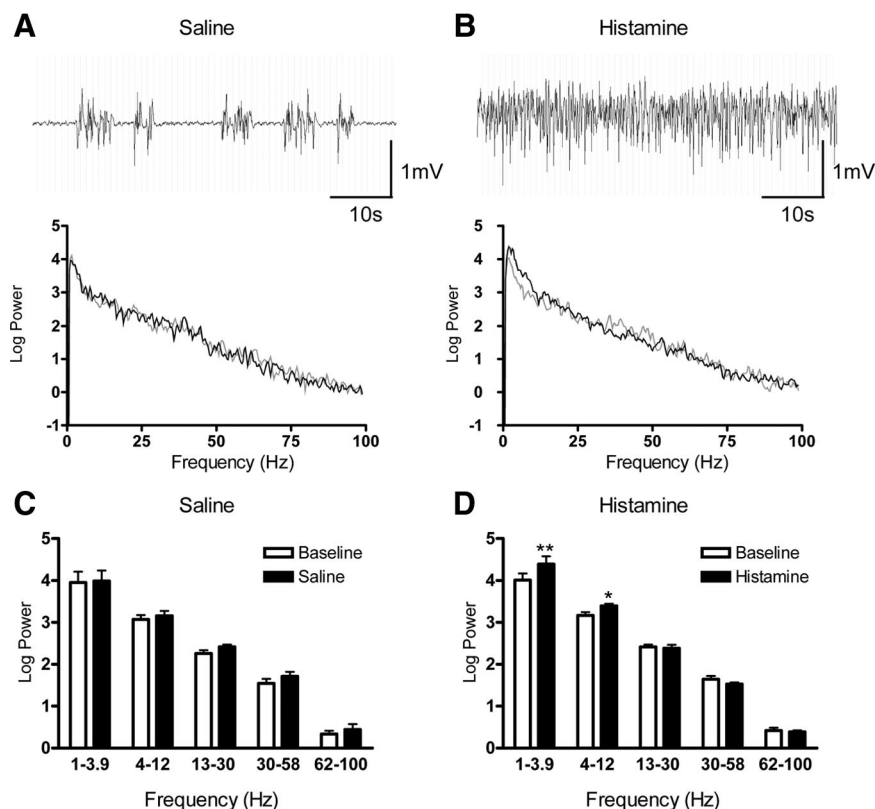
### *Administration of Histamine to NBM Induces Electroencephalographic Activation*

The neocortical (frontal cortical) electroencephalography in the rat showed a desynchronized or low-voltage fast activity pattern during awake immobility (fig. 2A). The electroencephalographic pattern changed with increasing concentration of inhaled isoflurane (fig. 2B and D), illustrated in each case after 15 min of inhaling isoflurane at a particular concentration. The rats showed no spontaneous movement with isoflurane concentration at 1%. As compared with the awake immobile electroencephalography, 1% isoflurane increased electroencephalographic power at the delta frequency (1–4 Hz) range and decreased power at > 30 Hz (fig. 2B). Increasing isoflurane to 1.4% resulted in a burst suppression pattern; *i.e.*, high-amplitude burst activity alternating

with suppressed background activity (fig. 2C). Further increase of isoflurane to 2.1% led to a prevailing isoelectric activity of the electroencephalography, shown as a low-amplitude power spectrum across all frequencies (fig. 2D).

The effect of histamine on electroencephalographic activation was first examined under 1.4% isoflurane anesthesia. Figure 3 illustrates two experiments from the same rat, first infused with vehicle (saline) bilaterally into the NBM, and then 4 days later with histamine bilaterally into the NBM. There was no change in the neocortical or hippocampal electroencephalographic pattern after saline infusion in the NBM, also confirmed by the electroencephalographic power spectra (fig. 3A and C). However, infusion of histamine (1  $\mu$ g/0.5  $\mu$ l) into the NBM produced a strong activation response in the neocortical electroencephalography, characterized by a shift of electroencephalography from burst suppression pattern to slow-wave ( $\delta$ ) activity (fig. 3B). This activation was observed within approximately 7 min after the start of histamine infusion. In all six rats tested, the neocortical electroencephalography did not return

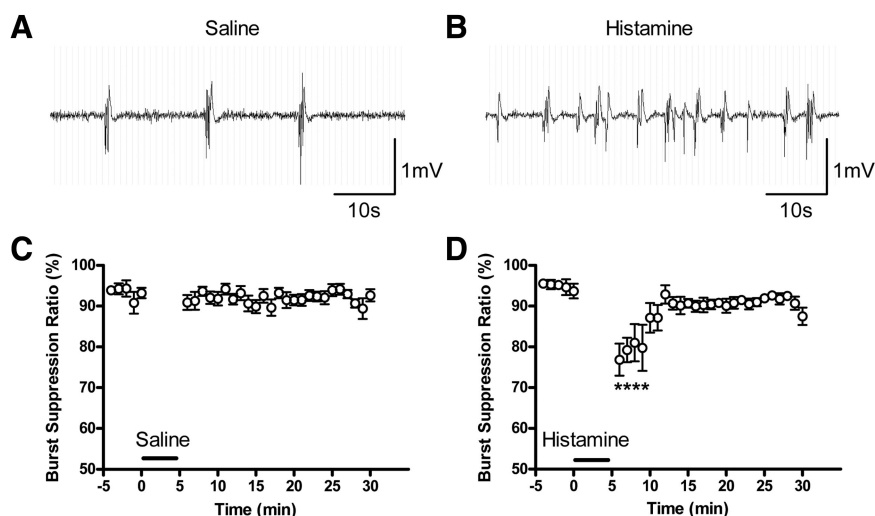
**Fig. 3.** Nucleus basalis magnocellularis (NBM) administration of histamine changes electroencephalographic burst suppression pattern to  $\delta$  activity at moderate (1.4%) isoflurane anesthesia. (A and B) Representative electroencephalographic traces and corresponding power spectra in a rat infused with 0.5  $\mu$ l saline (A) or 1  $\mu$ g/0.5  $\mu$ l histamine (B) into the NBM. (C and D) Electroencephalographic power spectral analysis showed no effect of saline (C) in  $\delta$ ,  $\theta$ ,  $\beta$ , and  $\gamma$  frequency bands, whereas a small increase in  $\delta$  and  $\theta$  power was observed with histamine (D). Data are mean  $\pm$  SEM,  $n = 6$  per group. \* $P < 0.05$ , \*\* $P < 0.01$ , versus baseline, paired  $t$  test. Calibration of 1 mV and 10 s apply to electroencephalographic traces. The power spectrum in (A) and (B) postinfusion (black dark trace) is overlaid on the preinfusion spectrum (light gray trace). The power spectra were plotted in logarithmic units, with calibration of 4.27 log units = 0.5 mV peak-to-peak sine wave.

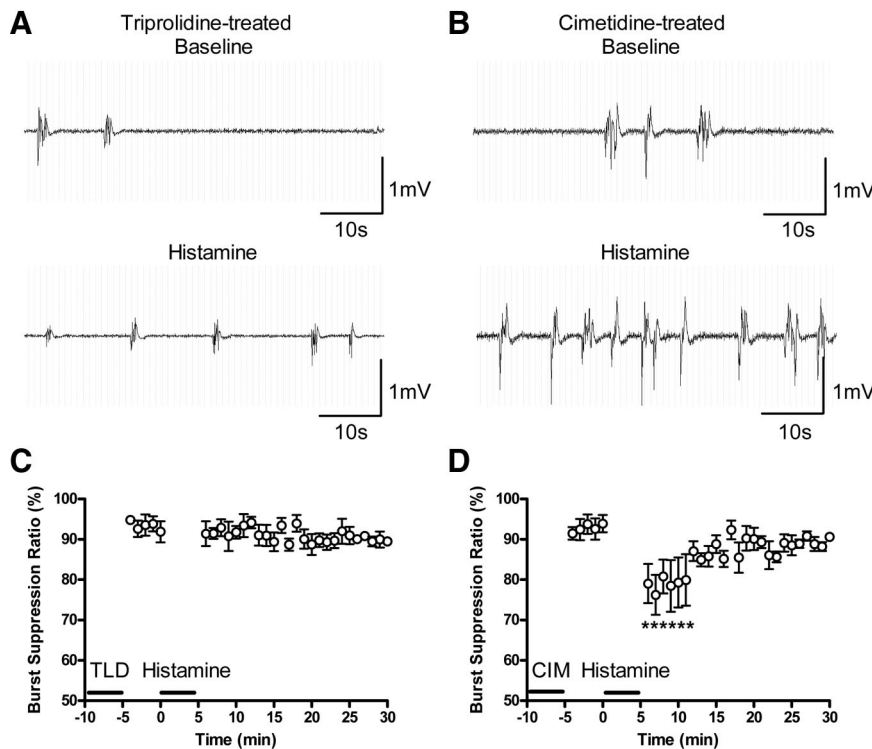


to the preinfusion baseline level, even at 25 min after histamine. Power spectral analysis showed that, as compared with baseline, NBM infusion of histamine increased the neocortical electroencephalographic power within the  $\delta$  (1–3.9 Hz) and  $\theta$  (4–12 Hz) frequency bands ( $P < 0.01$  and  $P < 0.05$ , respectively;  $n = 6$ ; paired  $t$  test; fig. 3D). Hippocampal electroencephalography also showed an increase in  $\delta$  power after histamine infusion in the NBM, with no change in the  $\theta$  electroencephalographic power (data not shown). Neocortical (and hippocampal) electroencephalographic power in the  $\beta$  (13–30 Hz) and  $\gamma$  (30–100 Hz) frequency bands was not significantly affected by histamine at 1.4% isoflurane.

At clinically relevant anesthetic concentrations, isoflurane was found to dose-dependently increase the burst-suppression ratio.<sup>32</sup> To further confirm and quantify histamine's effect on electroencephalographic activation, histamine or saline was infused into the NBM of rats under 2.1% isoflurane anesthesia. The average preinfusion BSR was similar in the saline and histamine experiments ( $93.20 \pm 1.27\%$  for saline and  $93.70 \pm 1.80\%$  for histamine,  $P > 0.05$ ,  $n = 8$ ). Histamine (1  $\mu$ g/0.5  $\mu$ l), but not saline, infused into the NBM induced a significant decrease in BSR (fig. 4). Two-way ANOVA revealed a significant drug effect ( $F[1,420] = 24.13$ ,  $P < 0.0001$ ,  $n = 8$ ), time effect ( $F[29,420] = 3.57$ ,  $P < 0.0001$ ,  $n =$

**Fig. 4.** Nucleus basalis magnocellularis (NBM) administration of histamine decreased the burst suppression ratio (BSR) during deep (2.1%) isoflurane anesthesia. (A and B) Representative electroencephalographic traces in a rat infused with 0.5  $\mu$ l saline (A) or 1  $\mu$ g/0.5  $\mu$ l histamine (B) into the NBM. (C and D) The BSR plotted versus time, with zero time as the onset of saline (C) or histamine (D) infusion. Data are mean  $\pm$  SEM,  $n = 8$  per group. \* $P < 0.05$ , post hoc comparison between the saline and histamine groups, after a significant two-way ANOVA.





**Fig. 5.** Histamine-induced electroencephalographic activation was blocked by the H1 receptor antagonist, but not by the H2 receptor antagonist. (A) Representative electroencephalographic traces showing nucleus basalis magnocellularis (NBM) infusion of 5 µg/1 µl tripolidine (TLD) 10 min before histamine blocked the effect of histamine 1 µg/0.5 µl on burst suppression ratio (BSR). (B) Representative electroencephalographic traces showing NBM infusion of 25 µg/1 µl cimetidine (CIM) 10 min before histamine failed to block the effect of 1 µg/0.5 µl histamine on BSR. (C and D) The burst suppression ratio plotted versus time, with -10 min as the onset of tripolidine (C) or cimetidine (D) infusion and 0 min as the onset of histamine infusion. Data are mean ± SEM,  $n = 7-8$  per group. \* $P < 0.05$ , *post hoc* comparison between the cimetidine group and the cimetidine followed by the histamine group, after a significant two-way ANOVA.

8), and drug  $\times$  time interaction ( $F[29,420] = 2.87$ ,  $P < 0.0001$ ,  $n = 8$ ). *Post hoc* comparisons showed a significant difference between the saline and histamine groups at 6–9 min after the start of histamine infusion. The changes of the hippocampal electroencephalographic BSR induced by histaminergic agents were similar to those of the neocortical electroencephalography, so only data from cortical electroencephalography were presented.

#### *H1 but Not H2 Receptor Antagonist Delivered to the NBM Antagonized the Electroencephalographic Activation Elicited by Histamine*

Antagonists of H1 and H2 receptors were infused directly into the NBM to reveal the specific receptor underlying the electroencephalographic activation effect of histamine (fig. 5). Under 2.1% isoflurane, NBM infusion of H1 (5 µg/1 µl tripolidine) or H2 (25 µg/1 µl cimetidine) receptor antagonist did not significantly change the baseline BSR. However, when pretreated with tripolidine, histamine (1 µg/0.5 µl) failed to induce a significant change in the BSR of the electroencephalography (fig. 5, A and C). Two-way ANOVA showed no significant drug effect for histamine when pretreated with tripolidine, as compared to tripolidine alone ( $F[1,390] = 0.003$ ,  $P > 0.05$ ,  $n = 7$  and 8), suggesting that tripolidine attenuated the electroencephalographic activation in response to histamine. On the other hand, pretreatment with the H2 antagonist cimetidine did not affect the histamine-induced suppression of BSR (fig. 5, B and D). The main effect of cimetidine and then histamine, as compared

with cimetidine alone, was significant ( $F[1,390] = 169.49$ ,  $P < 0.0001$ ;  $n = 7$  and 8, two-way ANOVA). These results indicate that histamine-induced electroencephalographic activation during isoflurane anesthesia was mainly mediated by H1 receptors in the NBM.

#### *NBM Administration of Histaminergic Agents Modulated Respiratory Rate and Emergence from Isoflurane Anesthesia*

Histamine produced a small but statistically significant increase in respiratory rate at 5 min after NBM infusion during 2.1% isoflurane anesthesia ( $P < 0.05$  or  $P < 0.001$ , Bonferroni's *post hoc* test *vs.* other groups, one way ANOVA,  $n = 8$ ; fig. 6A). However, tripolidine or cimetidine, as compared with saline, administered to the NBM induced no significant changes in respiratory rate ( $P > 0.05$ , Bonferroni's *post hoc* test after one way ANOVA,  $n = 8$ ; fig. 6A).

Emergence from anesthesia was defined as regaining the righting reflex. Emergence was associated with a low-voltage fast-frequency (desynchronized) electroencephalography, and this electroencephalographic pattern was similar among all groups. After 60 min exposure to 2.1% isoflurane, the time to recover the righting reflex (emergence time, Fig. 6B) was significantly reduced in rats infused with histamine (1 µg/0.5 µl) in the NBM, as compared with those infused with saline in the NBM ( $11.59 \pm 2.12$  min for saline group *vs.*  $4.04 \pm 0.49$  min for histamine group,  $P < 0.05$  by one-way ANOVA with Bonferroni's *post hoc* test,  $n = 8$ ). In contrast, rats infused with the H1 receptor antagonist tripolidine (5



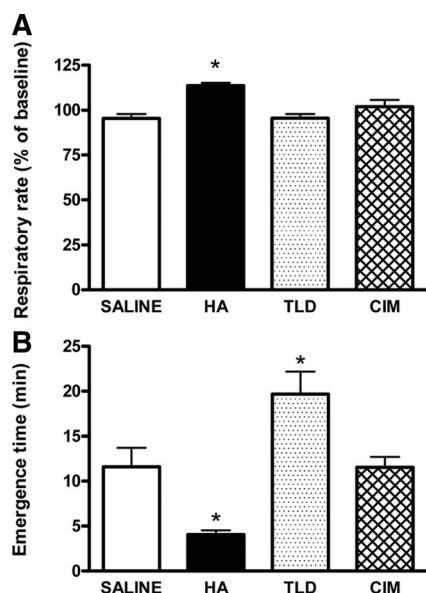


Fig. 6. Effect of nucleus basalis magnocellularis (NBM) administration of histaminergic drugs on respiratory rate and emergence from isoflurane anesthesia. (A) Changes in respiratory rate at 5 min after NBM infusion of a histaminergic drug. (B) Emergence from anesthesia determined by the time required for recovery of righting reflex after isoflurane was discontinued. All rats were exposed to 60 min of 2.1% isoflurane; saline (0.5  $\mu$ l), histamine (HA, 1  $\mu$ g/0.5  $\mu$ l), triprolidine (TLD, 5  $\mu$ g/1  $\mu$ l) or cimetidine (CIM, 25  $\mu$ g/1  $\mu$ l) was infused into the NBM 30 min before the cessation of isoflurane delivery. Data are mean  $\pm$  SEM,  $n = 8$  per group. \* $P < 0.05$  versus saline treatment (one-way ANOVA and Bonferroni test).

$\mu$ g/1  $\mu$ l) in the NBM required a significantly longer time to regain the righting reflex ( $19.69 \pm 2.48$  min), as compared with rats with saline infused in the NBM ( $P < 0.05$ , one-way ANOVA with Bonferroni's *post hoc* test,  $n = 8$ ). H2 receptor antagonist cimetidine (25  $\mu$ g/1  $\mu$ l) infused in the NBM had no significant effect on the emergence time ( $11.52 \pm 1.18$  min), as compared with saline-treated rats ( $P > 0.05$ , one-way ANOVA with Bonferroni's *post hoc* test,  $n = 8$ ).

## Discussion

The results of this study showed that acute administration of histamine into the NBM of the basal forebrain induced electroencephalographic activation, increased respiratory rate, and accelerated emergence from isoflurane anesthesia. In contrast, administration of the H1 receptor antagonist blocked the electroencephalographic activation induced by histamine and delayed emergence from anesthesia. The study suggests a possible role of the basal forebrain histaminergic pathway in modulating arousal/emergence from isoflurane anesthesia.

Electroencephalography is a continuous, noninvasive method that has been used as a measure of anesthetic drug action on the central nervous system.<sup>33</sup> In the current study, a stable steady-state electroencephalographic pattern was observed after 15 min administra-

tion of isoflurane at a particular concentration. In line with previous findings, the effects of isoflurane-induced electroencephalographic slowing, burst suppression, and isoelectric activity were concentration dependent.<sup>34</sup> At 2.1% isoflurane, infusion of histamine into the NBM decreased the BSR. At 1.4% isoflurane, histamine shifted the neocortical electroencephalography from a burst suppression pattern to  $\delta$  activity, with power that spilled into the adjacent  $\theta$  frequency band. This evidence indicates that histamine, when applied into the NBM, can modulate electroencephalographic activity during isoflurane anesthesia.

Histamine-induced electroencephalographic activation was greatly attenuated by triprolidine but not by cimetidine, suggesting that the cortical activation effect of histamine involved H1 receptors in the NBM. Activation of H1 receptors leads to a depolarization and/or an increase in the firing frequency of neurons.<sup>6</sup> In the basal forebrain, a clear interaction has been demonstrated between the central histaminergic and cholinergic pathways. *In vitro* study revealed that histamine could depolarize NBM cholinergic cells mainly through H1 receptors in basal forebrain slices.<sup>17</sup> Moreover, microdialysis of histamine or H1 receptor agonist into the basal forebrain resulted in increased release of acetylcholine from the neocortex.<sup>18</sup> Histamine was also found to excite noncholinergic cells, including the  $\gamma$ -aminobutyric acid-mediated and glutamatergic neurons in the basal forebrain.<sup>35,36</sup> Thus, histamine likely influenced neocortical electroencephalographic activity by action on both cholinergic and noncholinergic cells in the NBM. Furthermore, histamine is known to activate N-methyl-D-aspartic acid receptors, which has been implicated in the induction of burst suppression by isoflurane.<sup>34,37</sup>

In addition to electroencephalographic activation, histamine application into the NBM significantly increased the rate of respiration, suggesting a behaviorally arousing effect. H1 receptor antagonist triprolidine and H2 receptor antagonist cimetidine had no effect on the breathing rate during isoflurane anesthesia. The lack of effect on breathing rate by triprolidine or cimetidine could be because of a floor effect caused by the rapid and powerful depressant action of isoflurane on breathing rate. It is believed that histamine might affect the breathing pattern centrally *via* H1 receptors.<sup>38</sup>

Recent studies suggest that natural sleep and anesthesia may share similar neuronal pathways. Histamine release in the brain is strongly related to the sleep-wake cycle, with higher level of histamine during wake episodes than during sleep episodes.<sup>39</sup> Similarly, the release of histamine was significantly increased with a decrease in inhaled anesthetic concentration.<sup>40</sup> In this study, a facilitation of behavioral arousal was demonstrated after NBM infusion of histamine when the animals were allowed to emerge from anesthesia after administration of 2.1% isoflurane was stopped. This finding is consistent

with a previous report, which showed that intraventricular administration of high doses (5–25  $\mu$ g) of histamine decreased the duration of LORR in pentobarbital-anesthetized rats.<sup>41</sup> More importantly, we found that the time to emerge from isoflurane anesthesia was prolonged by NBM administration of the H1 receptor antagonist triprolidine. However, the same dose of triprolidine itself could not induce anesthesia or LORR in rats (data not shown). Considering that triprolidine would block the action of endogenous histamine at postsynaptic H1 receptors, we suggest that physiologic activation of the basal forebrain endogenous histaminergic pathway is involved in emergence from general anesthesia.

In addition to histamine, evidence also suggests that other neurotransmitters can modulate anesthesia response *via* actions on the wakefulness-promoting basal forebrain area. For example, orexin-A, a regulatory peptide that promotes wakefulness, administered in the basal forebrain induced signs of electroencephalographic arousal in rats during isoflurane anesthesia.<sup>42</sup> Adenosine, a putative sleep factor, can affect the anesthetic action of isoflurane *via* the neurons of the basal forebrain.<sup>43</sup> It has also been previously demonstrated that selective lesion of the cholinergic neurons in the NBM potentiated the anesthetic effects of propofol.<sup>20</sup> All these findings support the essential role of the basal forebrain as an important pathway for activating the cortex.<sup>44</sup>

While a number of studies in the past have attempted to describe the neural mechanism underlying anesthetic-induced unconsciousness, little attention has been paid to neural circuits responsible for emergence from anesthesia. A recent elegant study by Kelz *et al.*,<sup>45</sup> using genetic ablation of orexinergic neurons and a selective orexin-A receptor antagonist, demonstrated the role of the endogenous orexin system in the emergence from, but not the entry into, the anesthetized state. The arousal effect of orexin-A may depend on the activation of histaminergic neurotransmission *via* H1 receptors. Orexin infusion increases histamine release and wakefulness in normal but not in H1 receptor knockout mice.<sup>46</sup> To the best of our knowledge, this study presented the first evidence for the role of the basal forebrain as a possible neural locus, with the histaminergic pathway as a mechanism, in the emergence from anesthesia.

Our study has some limitations, the most important of which is that electroencephalographic activation during isoflurane anesthesia is not accompanied by a full behavioral arousal. Previous studies showed that behavioral reversal of anesthesia occurred at an anesthetic concentration only slightly above that causing LORR.<sup>47,48</sup> In this study, higher concentrations of isoflurane were used to study the electroencephalographic changes modulated by histaminergic agents. Despite the lack of full behavioral arousal from anesthesia, we did find that histamine administration in the NBM increased the respiratory rate,

which is a behavioral sign of a decreased depth of anesthesia. Histamine may not provide relief for all effects of isoflurane in the brain,<sup>49–51</sup> in particular in areas outside of the forebrain.<sup>52,53</sup> Second, the effect of histaminergic drugs on the induction of anesthesia was not examined in this study. Mammoto *et al.*<sup>40</sup> previously reported that systemic administration of an H1 antagonist decreased the anesthetic requirement for halothane evaluated as the minimum alveolar concentration, indicating that changes in histaminergic neuronal activities affect anesthetic requirement. Our finding, along with that of Mammoto *et al.*, suggests that endogenous histamine system might be essential to both the induction and emergence from general anesthesia. However, the exact role of the basal forebrain in the induction of anesthesia needs to be further studied.

In clinical practice, preoperative administration of antihistamines causes a delayed recovery of consciousness after anesthesia.<sup>54</sup> The results of this study suggest that histamine activation of H1 receptors in the NBM induces electroencephalographic arousal and facilitates emergence from isoflurane anesthesia. These findings support the hypothesis that the basal forebrain histaminergic pathway appears to play a role in modulating arousal/emergence from anesthesia.

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