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Short communication

Electrical stimulation of the parabrachial nucleus induces reanimation from isoflurane general anesthesia

Fanuel Muindi[a,c,*], Jonathan D. Kenny[a,b], Norman E. Taylor[a,b], Ken Solt[a,b], Matthew A. Wilson[a,c], Emery N. Brown[a,b,c,d,e], Christa J. Van Dort[a,b,c,**]

[a] Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, United States
[b] Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston, MA, United States
[c] Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, United States
[d] Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA, United States
[e] Harvard-Massachusetts Institute of Technology Division of Health Science and Technology, Massachusetts Institute of Technology, Cambridge, MA, United States

HIGHLIGHTS

- We tested the hypothesis that electrical activation of the glutamatergic parabrachial nucleus (PBN) in the brainstem is sufficient to induce reanimation (active emergence) during continuous isoflurane general anesthesia.
- Emergence from isoflurane anesthesia caused a selective increase in the number of active neurons in the lateral PBN.
- The electrical stimulation of the PBN induced behavioral arousal and restoration of the righting reflex.

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ABSTRACT

Clinically, emergence from general anesthesia is viewed as a passive process where anesthetics are discontinued at the end of surgery and anesthesiologists wait for the drugs to wear off. The mechanisms involved in emergence are not well understood and there are currently no drugs that can actively reverse the state of general anesthesia. An emerging hypothesis states that brain regions that control arousal become active during emergence and are a key part of the return to wakefulness. In this study, we tested the hypothesis that electrical activation of the glutamatergic parabrachial nucleus (PBN) in the brainstem is sufficient to induce reanimation (active emergence) during continuous isoflurane general anesthesia. Using c-Fos immunohistochemistry as a marker of neural activity, we first show a selective increase in active neurons in the PBN during passive emergence from isoflurane anesthesia. We then electrically stimulated the PBN to assess whether it is sufficient to induce reanimation from isoflurane general anesthesia. Stimulation induced behavioral arousal and restoration of the righting reflex during continuous isoflurane general anesthesia. In contrast, stimulation of the nearby central inferior colliculus (CIC) did not restore the righting reflex. Spectral analysis of the electroencephalogram (EEG) revealed that stimulation produced a significant decrease in EEG delta power during PBN stimulation. The results are consistent with the hypothesis that the PBN provides critical arousal input during emergence from isoflurane anesthesia.

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Emergence from general anesthesia is viewed as a passive process whereby anesthetics are discontinued at the end of surgery. Currently, there are no drugs available to actively reverse general anesthesia. Numerous sites in the brain have been shown to promote arousal. These include acetylcholine neurons in the pedunculopontine and laterodorsal tegmental areas, orexin/hyprocretin neurons in the lateral hypothalamus, dopamine neurons in the ventral tegmental area, histamine neurons in the tuberomammillary nucleus, norepinephrine neurons in the locus

* Corresponding author at: Massachusetts Institute of Technology, 43 Vassar Street, Cambridge, MA 02446, United States.
** Corresponding author at: Massachusetts General Hospital, Harvard Medical School, 77 Massachusetts Avenue, 46–5227, Cambridge, MA 02139, United States.
E-mail addresses: fmuindi@mit.edu (F. Muindi), vandortc@mit.edu (C.J. Van Dort).

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ceruleus, and others [1]. The pharmacological activation of cholinergic [2], histaminergic [3], noradrenergic [4], and dopaminergic arousal pathways [5–8], and the orexin/hypocretin neurons in the lateral hypothalamus have been reported to produce varying arousal responses during general anesthesia [9]. However, only cholinergic and dopaminergic areas have been shown to induce reanimation (active emergence) from continuous general anesthesia.

The available data suggest that it is possible to induce active emergence from general anesthesia. However, our understanding of the involvement of the brain's arousal centers in emergence is incomplete. Recent evidence has implicated the parabrachial nucleus (PBN) in promoting arousal [10,11]. The majority of neurons in the PBN are glutamatergic [12] and project to numerous areas in the brain, including the basal forebrain, hypothalamus, thalamus, amygdala, and the cortex [13,14]. Altogether, the PBN is well

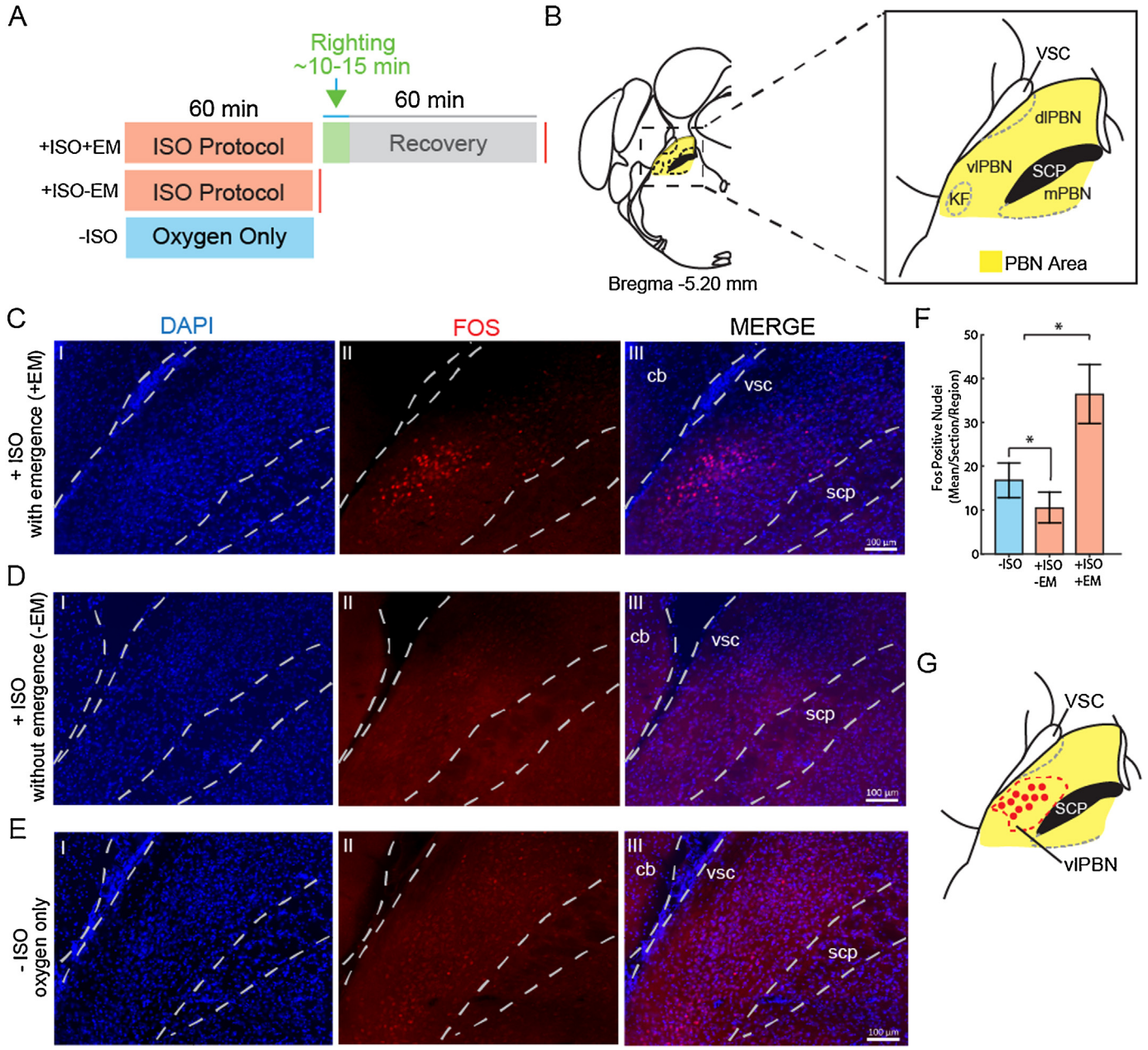


Fig. 1. c-Fos expression in the PBN after exposure to isoflurane with or without emergence. (A) Experimental protocol for quantifying the number of c-Fos positive nuclei in three groups of mice. The first group (+ISO + EM) underwent passive emergence from the isoflurane anesthesia protocol shown in A, and had return of righting before being sacrificed. The second group (+ISO – EM) underwent the same isoflurane anesthesia protocol and was sacrificed before emergence. The last group (-ISO) received only oxygen. (B) A coronal section representation showing the PBN area and local structures in mouse. (C) DAPI stained, c-Fos stained, and merged images from +ISO + EM mice. The c-Fos image shows a visible cluster of c-Fos positive nuclei in the lateral PBN. (D) DAPI stained, c-Fos stained, and merged images from +ISO-EM mice. c-Fos staining shows a comparative lack of positive nuclei. (E) DAPI stained, c-Fos stained, and merged images from awake mice that were only exposed to oxygen. (F) A coronal section schematic showing the PBN area as well as the superior cerebellar peduncle (SCP), the lateral PBN and accompanying c-Fos positive nuclei are shown as red circles. (G) Mean number of c-Fos positive nuclei for each section and region across the three groups of animals. The c-Fos positive nuclei count for the +ISO + EM group was significantly higher (*) than the +ISO-EM and –ISO groups. The c-Fos positive nuclei count for the –ISO group was also significatly higher than the +ISO-EM group. Error bars indicate 95% confidence intervals around the mean. *Abbreviations*: dorsolateral Parabrachial Nucleus, dlPBN; ventrolateral Parabrachial Nucleus, vlPBN; medial Parabrachial Nucleus, mPBN; Kolliker-Fuse Nucleus, KF; Ventral Spinocerebellar Tract, VSC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

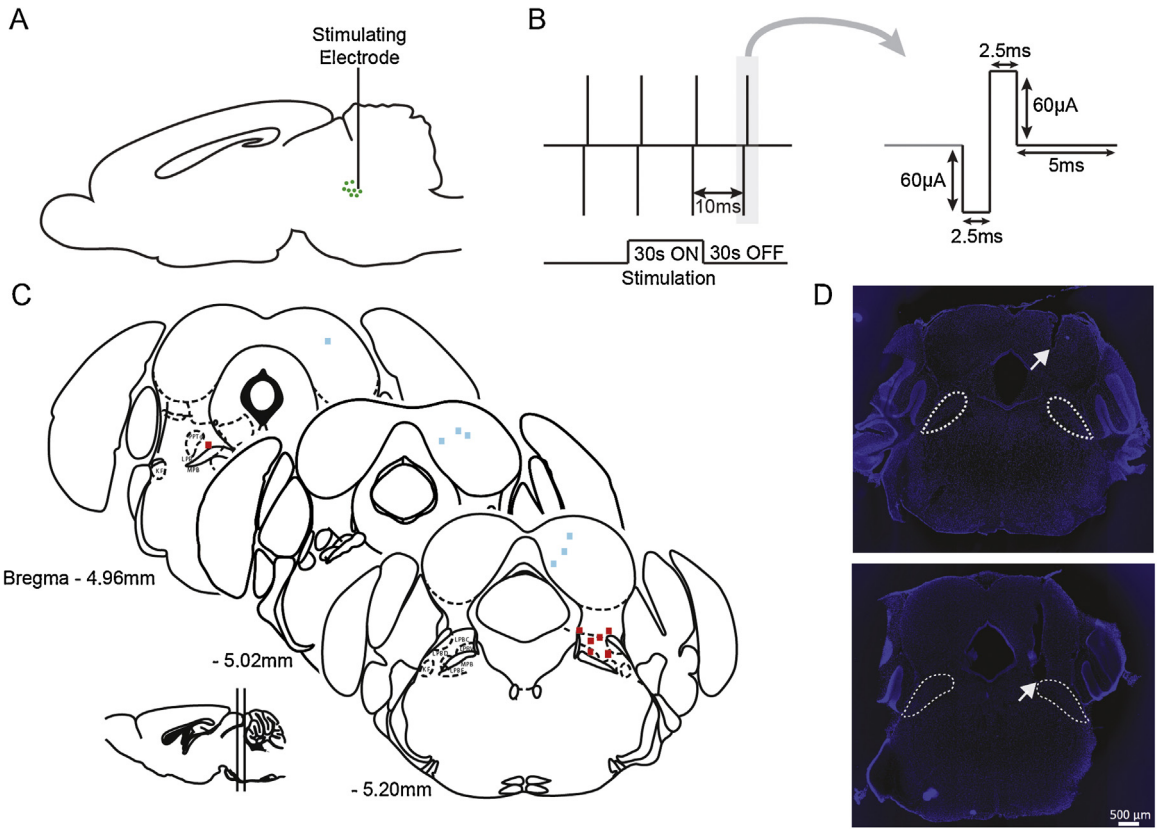


Fig. 2. Histological verification of electrical stimulation electrode placement in the PBN and CIC. (A) A sagittal secion of the mouse brain showing the approximate location of electrical stimulation electrodes in the PBN. (B) The current intensity was 60 μA and the stimulation frequency was 100 Hz. The stimulation duration was 30 s on/30 s off. (C) Coronal sections showing the histological placement of electrical stimulation electrodes in the PBN (red) and in the CIC (blue). A sagittal insert shows the location of the PBN. (D) DAPI stained coronal sections show the electrode tracts into the CIC (top image) and into the PBN (bottom image). Note that 2 of the electrode locations (anterior/posterior: −5.40 mm) reached the posterior PBN and thus are not shown in the diagram. Dashed white lines show the outline of the lateral and medial portions of the parabrachial nucleus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

positioned to be able to alter arousal levels. However, no studies to date have assessed the role of the PBN in emergence from general anesthesia.

Here, we tested the hypothesis that the PBN is active during emergence from anesthesia, and also assessed whether electrical stimulation of the PBN is sufficient to induce reanimation during continuous isoflurane general anesthesia. Animal studies were approved by the Committee on Animal Care, Massachusetts Institute of Technology, Cambridge, Massachusetts. Male C57-BL/6J adult mice 4–5 months (Jackson Laboratory) were kept on a standard day-night cycle (lights on at 7:00 AM, and off at 7:00 PM). We first assessed c-Fos expression in the PBN for the following three experimental groups: One group underwent the protocol for continuous isoflurane anesthesia (see Supplementary Material and Methods) and passively emerged before being sacrificed (+ISO + EM, n = 5). Another group underwent the same isoflurane anesthesia protocol and was sacrificed before emergence (+ISO-EM, n = 3). The last group was only exposed to oxygen before being sacrificed (-ISO, n = 2). The anesthesia protocol involved exposing mice to 2.5% isoflurane for 20 min, then placing the mice supine before reducing the isoflurane dose and maintaining it at 0.9–1.0% for the next 40 min. Additional details of the experiment procedures and statistical analysis are provided in the Supplementary Material and Methods.

Fig. 1A shows a summary of the c-Fos experiments. Fig. 1B and 1G are a representation of the PBN and local structures. DAPI and c-Fos expression are seen in Fig. 1C for mice that passively emerged from isoflurane anesthesia, and in Fig. 1D for mice that were sacrificed before emerging from isoflurane anesthesia. A large cluster of c-Fos positive nuclei in the ventrolateral portion of the PBN (vlPBN) can be seen in Fig. 1C for mice that emerged from isoflurane anesthesia. Fig. 1D shows a relative paucity of c-Fos positive nuclei in mice that did not emerge. Fig. 1E shows DAPI stained, c-Fos stained, and merged images from mice that were exposed to oxygen only.

Fig. 1F shows the counts of c-Fos positive nuclei (mean/section/region) grouped across mice for the three experimental protocols. The mean number of c-Fos positive nuclei (5–6 sections per mouse) for the −ISO group was 16.8 (95% CI: 12.8–20.8, 20 PBN regions; n = 2), for the +ISO-EM group was 10.4 (95% CI: 7.2-14; 29 PBN regions; n = 3), and for the +ISO + EM group was 36.4 (95% CI: 29.7–43.3, 44 PBN regions; n = 5). The c-Fos positive nuclei counts were significantly higher in the +ISO + EM group when compared to the +ISO-EM group (difference 25.9, 95% CI: 18.3–33.5) group and the −ISO group (difference 19.6, 95% CI: 11.9–27.5). Although small, a significant difference was detected between the −ISO group and +ISO − EM (difference 6.3, 95% CI: 1.15–11.5).

In a separate group of animals, we next assessed whether electrical stimulation of the PBN is sufficient to induce reanimation during continuous isoflurane general anesthesia. The encephalogram (EEG) was recorded from the frontal and parietal corticies (see Supplementary Material and Methods). Fig. 2A shows a sagittal section with the approximate location of stimulation electrodes in the PBN (n = 9). Fig. 2B shows the electrical stimulation protocol (30 s on/30 s off) used for both PBN and CIC stimulations. The stimulation intensity was fixed at a current of 60 μA and the frequency was 100 Hz. Fig. 2C shows a coronal section (adapted from the Mouse Brain Atlas [25]) with the location of PBN (red, n = 9) and

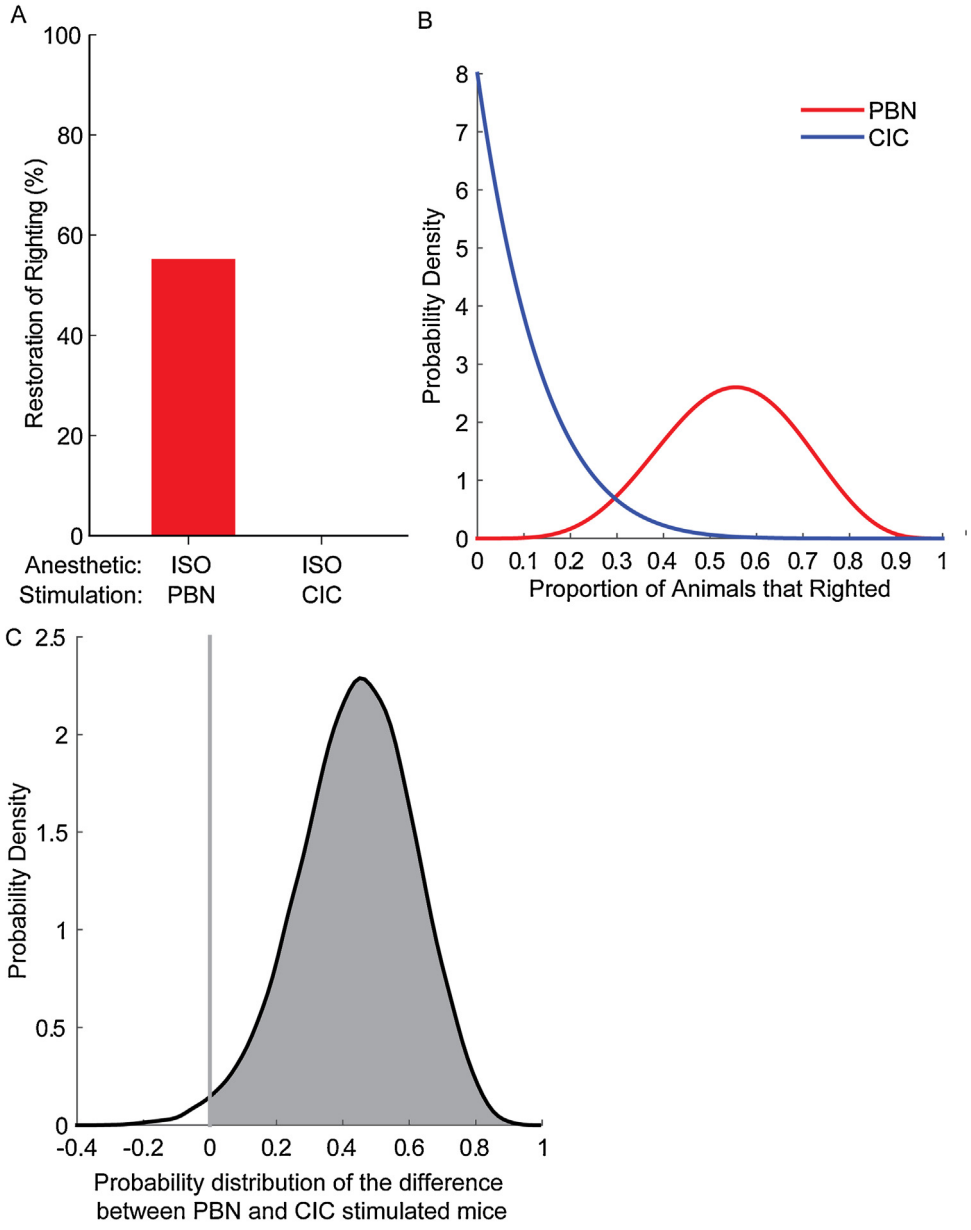


Fig. 3. Reanimation from continuous isoflurane anesthesia by electrical stimulation of the PBN. (A) 5 out of 9 mice had return of righting with PBN stimulation while 0 out of 7 mice had return of righting with CIC stimulation. (B) Posterior densities for the propensity of righting for PBN (red) and CIC (blue) mice. Posterior densities are drawn from beta distributions. The posterior probability of PBN stimulation causing restoration of righting being significantly greater than the CIC group is represented by the area of the PBN distribution that does not overlap with the CIC distribution. (C) Probability distribution of the difference between the red and blue curves shown in part B. [Pr(PBN > CIC) = 0.97]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CIC (blue, n = 7) stimulation electrodes as determined by histology. Fig. 2D shows two DAPI stained coronal sections with stimulating electrode tracts in the CIC (top image) and in the PBN (bottom image).

Righting was restored in 5 out of 9 mice that received electrical stimulation of the PBN during continuous isoflurane anesthesia, while righting was not observed in any of the 7 mice that received electrical stimulation of the CIC. PBN stimulation rapidly induced arousal responses in all of the animals during isoflurane general anesthesia which culminated in the return of righting in 5/9 mice (Fig. 3). Fig. 3A shows the results of these experiments. The beta distribution functions used to model the difference in propensity for return of righting are shown in Fig. 3B. Fig. 3C shows the probability distribution of the difference between the two curves shown in Fig. 3B. Bayesian 95% credibility intervals for the difference in propensity for righting between the PBN group and the CIC group were 0.07–0.75, and the posterior probability that the propensity for righting was higher in the PBN group than in the CIC group was 0.988, indicating that PBN stimulation caused a statistically significant increase in return of righting when compared to CIC stimulation.

The EEG recorded from an individual mouse during isoflurane anesthesia is shown in Fig. 4A. PBN stimulation (red line) caused a rapid decrease in EEG amplitude and increase in EEG frequency. The time-frequency domain spectrogram in Fig. 4B (computed from the EEG in Fig. 4A) shows a rapid loss of δ (0.1–4 Hz) power during the first 8 s of stimulation. Group comparisons of the power spectral densities from 30 s before stimulation (blue line) and 30 s during PBN stimulation (red line) can be seen in Fig. 4C. During PBN stimulation there was a significant decrease in δ power and an increase

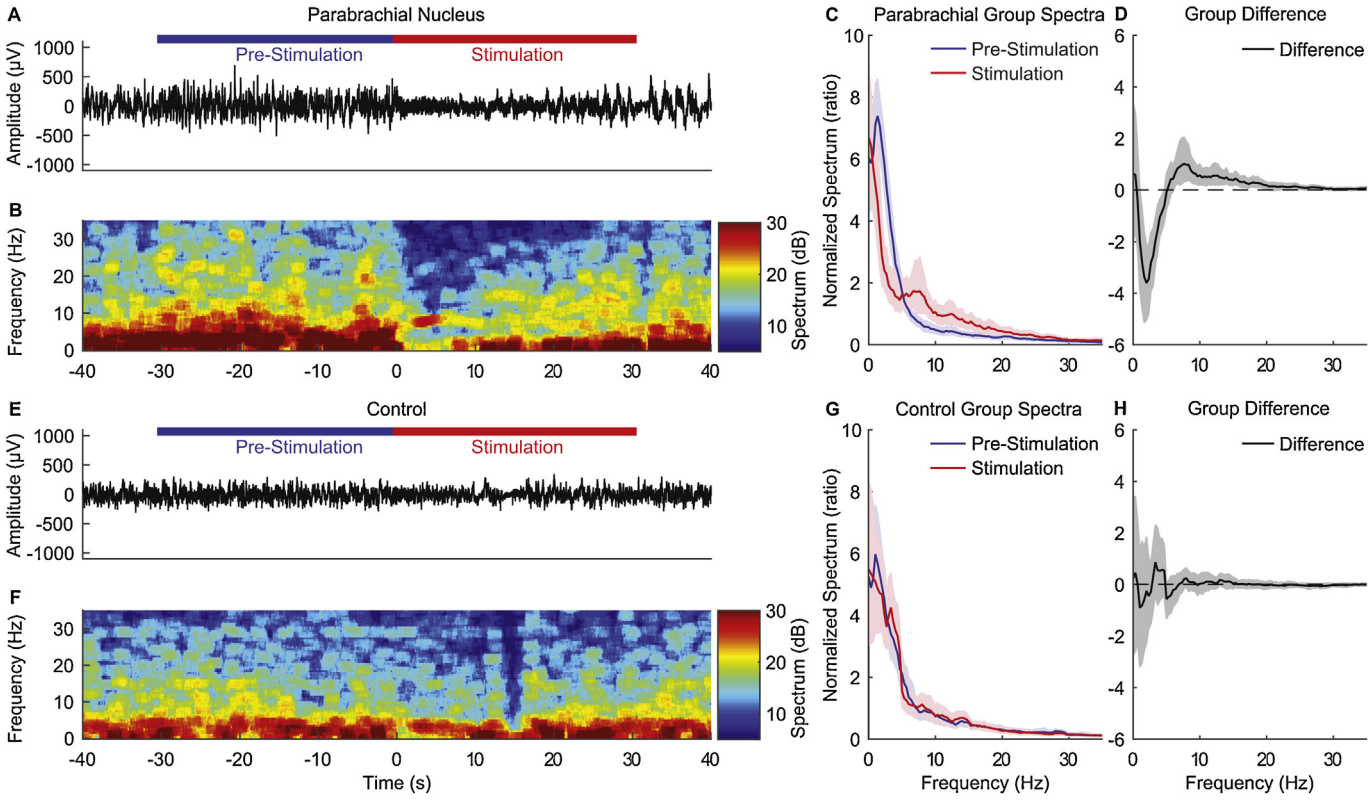


Fig. 4. EEG and spectral analysis of electrical stimulation of the PBN during continuous isoflurane anesthesia. (A) Representative 80-seconds of EEG from a mouse with time 0 indicating the beginning of the electrical stimulation during continuous isoflurane anesthesia. The blue and red lines indicate the 30-seconds used for the power spectral density comparison. EEG recorded from mice with electrodes in the PBN show an instantaneous decrease in amplitude, and increase in EEG frequency during electrical stimulation. (B) Time-frequency domain spectrogram computed from the EEG in A, warm colors indicate frequencies of high power while cool colors indicate frequencies of low power. A prompt loss in δ power can be seen during the stimulation around 0–8 s. (C) Normalized group power spectral densities from PBN animals with pre-stimulation (blue) and electrical stimulation (red), shaded areas indicate 95% confidence intervals. (D) Difference between stimulation and pre-stimulation power spectral estimates show a significant shift in peak power from θ to δ. (E) EEG recorded from a mouse with electrodes in the control area (CIC) shows less change in amplitude and frequency then the signal in A during electrical stimulation. (F) Time-frequency domain spectrogram computed from the EEG in D shows minimal loss in power during electrical stimulation. (G) Normalized group power spectral densities from control (CIC) animals with pre-stimulation (blue) and electrical stimulation (red), shaded areas indicate 95% confidence intervals. (H) No significant differences can be seen between pre-stimulation and stimulation power spectral densities as indicated by 95% confidence intervals overlapping with zero. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in θ (4–8 Hz) power which is indicative of cortical arousal [15,16]. The shaded areas represent 95% confidence intervals. In the PBN stimulation group, no spectral differences were observed between animals that had return of righting and those that did not right.

Fig. 4E shows the raw EEG during CIC electrical stimulation in an individual mouse. During CIC stimulation there was no change in EEG amplitude or frequency. The spectrogram in Fig. 4F shows a slight loss in δ power at the moment of stimulation. Fig. 4G shows the grouped comparisons between the pre-stimulation and stimulation power spectral densities for the CIC implanted mice. Overlapping 95% confidence intervals indicate no significant differences between the two spectral densities.

Our c-Fos immunohistochemical data reveals that the vlPBN is active during passive emergence from isoflurane general anesthesia (Fig. 1). Although the degree of change was small, significant decreases in c-Fos activity were observed between +ISO − EM when compared to −ISO. This decrease in an arousal promoting brain region during isoflurane, agrees with the results of Kelz et al. where isoflurane caused a large and significant decrease in c-Fos activity in another arousal-promoting area in the lateral hypothalamus which contains orexinergic neurons [9]. The large increase in c-Fos activity in the +ISO + EM group suggests that the PBN plays a key role in passive emergence from isoflurane anesthesia.

Several lines of evidence support the idea that increased neurotransmission from the PBN is important for arousal. It has been reported that lesions in the lateral and medial PBN in rats lead to increases in total sleep, mostly due to significant increases in NREM and REM sleep during the dark period [10,20]. Consistent with this result, selective deletion of the Vglut2 gene, which many PBN neurons express [12], has recently been shown to increase NREM sleep during the dark period [11]. Furthermore, single unit recordings in the lateral PBN of the cat across sleep and wake has revealed a division of neurons which exhibit (i) a decrease in firing during NREM sleep, (ii) an increase in firing during REM sleep and (iii) no change in firing across states relative to firing rates during wakefulness [21,22]. More importantly, the PBN has been shown to project to a number of areas in the brain involved in arousal and sleep including the basal forebrain, hypothalamus and also cortex [14]. Taken together, the data support the hypothesis that the PBN plays an important role in promoting cortical arousal.

In our study, this hypothesis is further supported by the demonstration of reanimation from general anesthesia in response to electrical stimulation of the PBN. PBN stimulation rapidly induced arousal responses in all 9 animals during isoflurane general anesthesia, which culminated in the return of righting in 5/9 mice. The behavioral arousal was accompanied with a prominent decrease in δ power during stimulation and an increase in θ power (Fig. 4) which is consistent with an arousal response during isoflurane general anesthesia. Importantly, electrical stimulation of the nearby CIC, which contains both GABAergic and glutamatergic neurons [23], did not induce righting in any of the animals, and no significant EEG changes were observed.

This result adds to the growing body of evidence suggesting that arousal-promoting pathways are involved in emergence from general anesthesia. Thalamic microinjections of nicotine in rats have been shown to induce reanimation during continuous sevoflurane anesthesia [2]. Norepinephrine infusion into the nucleus basalis elicited both behavioral and EEG arousal in desflurane-anesthetized rats, although restoration of righting was not reported [4]. More recently, it has been shown that electrical stimulation of the ventral tegmental area elicits reanimation in rats anesthetized with propofol and isoflurane [8]. Together, the current findings and previous work suggest that certain arousal pathways may be stimulated to induce reanimation from general anesthesia. Pharmacological targeting such arousal systems provides a promising new approach to promote rapid recovery from general anesthesia in surgical patients.

The current study has some limitations. c-Fos immunohistochemistry has low temporal resolution providing only a one-time view of neural activity in a given area. It is possible that the observed increases in c-Fos activity after emergence were related to changes in respiration [17], thermosensation [18,19], or cardiac activity [24]. Nevertheless, our study encourages further work to better understand how neural activity in the PBN affects the transition from the unconscious state to the conscious state during emergence from general anesthesia. In order to show definitively that glutamate neurons are driving the arousal effect, more selective techniques such as optogenetics that allow targeting of specific neuronal subtypes are necessary.

In summary, our data suggest that the lateral PBN is active in mice during passive emergence from isoflurane general anesthesia. In addition, electrical stimulation of the PBN is sufficient to induce reanimation from continuous isoflurane general anesthesia. We propose that the PBN provides critical arousal inputs to the cortex during emergence from isoflurane general anesthesia. Activating this arousal circuit may provide a novel method to enhance recovery from general anesthesia in surgical patients.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2016.03.021.

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