

Vascular and Neuronal Effects of General Anesthesia on Functional Magnetic Resonance Imaging

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Abstract

A number of functional magnetic resonance imaging (fMRI) studies rely on application of anesthetic agents that can modulate and complicate interpretation of the measured hemodynamic blood oxygenation level-dependent (BOLD) response. The purpose of this study was to investigate the effect of general anesthesia on two main components of BOLD signal including neuronal activity and vascular response. Breath-holding (BH) fMRI was conducted in wakefulness and under anesthesia states in patients with drug resistant epilepsy (DRE) who needed to get scanned under anesthesia during laser interstitial thermal therapy (LITT). BOLD and BOLD-cerebrovascular reactivity (BOLD-CVR) maps were compared between two states to assess the effect of anesthesia on neuronal activity and vascular factors. Overall, our findings revealed an increase in BOLD-CVR and decrease in BOLD response under anesthesia. The results proposed that the modulatory mechanism of anesthetics on neuronal and vascular components of BOLD signal may work in different ways. To our knowledge, this is the first human study to examine the effect of general anesthesia using BH fMRI imaging, enhancing our understanding of the effect of anesthesia on neuronal and vascular aspects of BOLD response and assisting the implication of general anesthesia and interpretation of outcomes in clinical setting.

Introduction

Blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) has been widely used in detection and mapping of brain function. However, it remains important to point out that BOLD response does not directly reflect neuronal activity but rather reflects cerebral hemodynamics alterations caused by neuronal activity. In fact, BOLD MRI is representative of the complex relationship between cerebral blood flow (CBF), oxygen metabolism, and neurovascular function^{1,2}. Even though the empirical coupling relationships between BOLD and CBF in healthy human subjects under conventional scanning scenarios is assumed to be intact, understanding of this relationship is essential because of the confounding factors that potentially alter neurovascular coupling^{3,4}. Recently, several studies have estimated the relationship between hemodynamic responses and neuronal activity in animal models. One potential confounding factor involved in these studies is the application of anesthesia. Anesthetic agents have been used in fMRI studies to limit potential artifacts induced by subject movement and to control the physiological factors. At the same time, anesthetics can depress spontaneous and evoked neuronal activity, alter both neuronal activity and vascular response, and modulate neurovascular coupling. However, the degree and dynamics of these effects depend on the type and dose of the anesthetics⁵⁻⁷.

To date, animal studies have demonstrated that BOLD response decreases in anesthetized animals compared to an awake state, presumably reflecting decreased neuronal excitation under anesthesia^{6,8}. However, sensitivity of brain regions to the anesthetic agents is divergent that may result in temporal variability of BOLD response in terms of its magnitude and spatial extent over time^{6,8,9}. Furthermore, it has been shown that volatile anesthetics such as sevoflurane have intrinsic dose-dependent vasodilatory effect that can complicate the interpretation of BOLD responses^{4,10,11}. In fact, the vasodilatory effect of

volatile anesthetics causes pial dilation, increases partial pressure, and elevates CBF. However, the degree of alteration in CBF varies according to the concentration and type of the agent. In fMRI studies including anesthetic agents, the brain vasculature responds to two factors including fMRI task stimulation and anesthesia that complicates the interpretation of BOLD response. As such, in these studies it is crucial to discriminate between vascular effects of the anesthetic agents and neuronal activity before one can properly interpret the associated findings^{9,12,13}.

Cerebrovascular reactivity (CVR) mapping has been used to detect the ability of cerebral vessels dilation or constriction and to estimate hemodynamic response to a vasodilatory challenge such as hypercapnia¹⁴⁻¹⁶. As such, breath-hold (BH) fMRI task has been proposed as a non-invasive, convenient approach that can be employed to map vascular reactivity response¹⁷⁻²⁴. CVR measurements have been performed under anesthesia in MRI-based animal studies; and using non-MRI-based approaches in humans. The present study is the first to conduct BH fMRI acquisition to evaluate the effect of general anesthesia on BOLD and BOLD-CVR responses. We aimed to quantify the effect of a volatile anesthetic agent on BOLD and BOLD-CVR brain maps in order to better understanding of the neuronal and vascular effects of anesthetic agents in human subjects.

Results

-Cerebrovascular reactivity (CVR)

There were significant differences in magnitude of BOLD-CVR obtained across gray matter and thalamus between two states of awake and under anesthesia. BOLD-CVR was generally higher under anesthesia than in awake state, particularly in the right middle and superior temporal gyrus, left middle and superior frontal gyrus, right caudate, left and right anterior cingulate, and cerebellum. In a few clusters, mainly located in occipital lobe, BOLD-CVR was lower under anesthesia ($p < 0.05$) (Figure 3). The list of significant clusters is shown in Table 1 where positive and negative regression coefficients (beta-weights) correspond with higher and lower group differences in BOLD-CVR between the two states, respectively. The contents of the table are reported based on TT-Daemon atlas provided in AFNI package.

Table 1

Brain clusters with significant group differences in BH BOLD-CVR response between awake and under anesthesia states based on TT-Daemon atlas provided by AFNI (t-test, p-corrected < 0.05, minimum cluster size of 150 voxels).

Cluster	Regions	Regression Coefficient (b-value)	Cluster size (voxels)	Volume (mm ³)	Coordination (Cmass) (x, y, z)
Cluster1	Left Cerebellum	-4.4	3012	16142.43	(29.1, 54.8, -25.8)
Cluster2	Left Anterior Cingulate Left Medial Frontal Gyrus	-1.2	351	1881.14	(7.5, -28.2, -5.4)
Cluster3	Right Caudate Right Anterior Cingulate	-1.4	340	1822.18	(-6.4, -5.2, -4.0)
Cluster4	Left Middle Occipital Gyrus Left Lingual Gyrus	2.3	319	1709.64	(23.1, 91.6, 5.2)
Cluster5	Right Lingual Gyrus	-0.63	231	1238.01	(-16.3, 53.7, -7.7)
Cluster6	Right Cerebellum	-3.11	224	1200.5	(-41.0, 46.8, -34.5)
Cluster7	Right Superior Temporal Gyrus Right Parahippocampal Gyrus	-1.2	211	1130.82	(-34.8, -3.7, -14.4)
Cluster8	Right Middle Occipital Gyrus Right Inferior Occipital Gyrus	1.65	174	932.53	(-36.7, 84.2, -1.8)
Cluster9	Right Middle Temporal Gyrus Right Middle Occipital Gyrus	1.1	173	927.17	(-43.8, 75.1, 12.6)
Cluster10	Left Middle Frontal Gyrus Left Superior Frontal Gyrus	-1.9	169	905.73	(21.7, -49.3, -9.0)

-BH BOLD group analysis

BOLD response group differences between awake and anesthetized states showed greater BOLD in awake compared to anesthetized state located in the frontal, temporal, parietal, and insular lobes; as well as the postcentral, and precentral gyrus, and thalamus ($p < 0.05$). The main clusters showing significant differences between awake and under anesthesia are shown in Figure 4. The list of significant clusters is represented in Table 2.

Table 2

Brain clusters with significant group differences in BH BOLD-CVR response between awake and under anesthesia states based on TT-Daemon atlas provided by AFNI (t-test, p-corrected < 0.05, minimum cluster size of 150 voxels).

Cluster	Regions	Regression Coefficient (b-value)	Cluster size (voxels)	Volume (mm ³)	Coordination (Cmass) (x, y, z)
Cluster1	Right Precentral Gyrus Right Middle Frontal Gyrus	8.43	1015	5439.76	(-20.5, 2.2, 55.7)
Cluster2	Right Culmen Right Fusiform Gyrus	34.20	697	3735.48	(-36.9, 53.1, -16.9)
Cluster3	Left Inferior Parietal Gyrus Left Supramarginal Gyrus	14.19	520	5786.87	(55.1, 38.5, 25.4)
Cluster4	Right Inferior Parietal Lobule Right Postcentral Gyrus	7.83	461	2470.67	(-47.4, 36.3, 42.3)
Cluster5	Right Middle Occipital Gyrus Right Middle Temporal Gyrus	7.44	342	1832.90	(-48.9, 62.6, -3.8)
Cluster6	Right Insula Right Precentral Gyrus	7.07	305	1634.60	(-43.3, -10.6, 5.8)
Cluster7	Left Medial Frontal Gyrus Left Superior Frontal Gyrus	4.38	287	1447.03	(4.1, -6.7, 49.9)
Cluster8	Left Cerebellum	6.43	270	1447.03	(37.0, 41.0, -22.5)
Cluster9	Right Middle Temporal Gyrus Right Superior Temporal Gyrus	7.53	236	1264.81	(-55.5, 40.5, 5.0)

Cluster	Regions	Regression Coefficient (b-value)	Cluster size (voxels)	Volume (mm ³)	Coordination (Cmass) (x, y, z)
Cluster10	Right Superior Frontal Gyrus Right Middle Frontal Gyrus	13.72	195	1045.07	(-27.9, -36.7, 36.0)
Cluster11	Left Precentral Gyrus Left Middle Frontal Gyrus	12.05	191	1023.64	(34.0, 9.8, 55.0)
Cluster12	Right Thalamus Right Caudate	5.60	180	964.68	(-10.4, 14.4, 10.2)
Cluster13	Left Insula	9.09	174	932.53	(33.1, -17.1, 6.3)
Cluster14	Right Inferior Parietal Lobule Right Superior Temporal gyrus	5.31	169	905.73	(-55.6, 35.5, 23.8)
Cluster15	Right Middle Frontal Gyrus Right Superior Frontal Gyrus	12.15	151	809.26	(-42.3, -43.0, 16.1)

-Breath holding BOLD vs. Breath holding BOLD-CVR

The average of BOLD response was extracted from the three distinct masks as mentioned above. *3dmaskave* in AFNI was used to measure the mean and standard deviation (SD) of BOLD from each mask. The average and SD of BOLD response were lower under anesthesia compared to awake state using all three masks. The statistical t-test revealed non-significant p-values comparing two groups ($p > 0.05$) (Figure 5). Additionally, Levene's test showed higher between-subject variabilities in awake compared to anesthetized state for both BOLD-CVR and BOLD responses ($p < 0.05$).

Discussion

This study employed BH fMRI challenge in both awake and under general anesthesia states to help parse out the contributions of neuronal and vascular effects of general anesthesia. Propofol and sevoflurane, the most common intravenous and inhaled anesthetics agents have been used in neurosurgical anesthesia in human studies. However, the effect of these agents on cerebrovascular hemodynamic

response and during BH fMRI has not been clearly determined. It has been shown that anesthetic agents cause physiological alteration that can lead to hemodynamic BOLD response changes including alteration in neurovascular coupling, neuronal activity, and vascular reactivity. Hence, for interpretation of the results, this is requisite to contemplate pharmacology and physiological impact of the anesthetics that used in the study ^{4,6}.

-Effects of Anesthesia on Cerebral Vasculature

Our findings demonstrated that BOLD-CVR increased under anesthesia compared to awake state. We speculate that the increase in BOLD-CVR is linked with the physiological characteristic of the anesthetics. Anesthetic agents have been shown to affect the dynamics of the cerebral vasculature via direct action on the receptors of vascular smooth muscles that results in cerebral vasodilation. In fact, volatile anesthetics such as sevoflurane have been known to possess intrinsic vasodilatory activity due to their direct effect on vascular smooth muscles in a dose-dependent manner ^{10,25-27}.

In a recent study Sakata et al. 2019 showed that in anesthetized rats, sevoflurane induced greater vasodilatory effects under hypercapnia. They concluded that cerebrovascular response to hypercapnia is higher in patients anesthetized with volatile agents ¹². Additionally, a study on patients undergoing spine surgery showed that sevoflurane has vasodilatory effect on cerebral arteries ¹⁰. Similarly, the previous study by Wang et al. 2017 on patients undergoing laparoscopic surgery found that while propofol has a vasoconstrictive effect, CVR increased due to the dominant vasodilatory impact of sevoflurane. The authors concluded that in patients with impaired cerebrovascular reserve capacity, application of inhaled anesthetics can be taken into account in order to compensate vasodilation ability of cerebral vessels that leads to attenuation of the cerebrovascular resistance to partial pressure of carbon dioxide (PaCO₂) ¹¹.

However, the effect of the general anesthetics on cerebral vessels remains controversial, as they may cause both vasodilation or vasoconstriction depending on the type and dose of the agent ²⁵⁻²⁷. Several human studies reported no significant change in BOLD-CVR under anesthetic with sevoflurane ²⁸. Juhász et al. 2019 used a combination of propofol and sevoflurane during induction and maintenance phases in ventilated patients and found an initial increase of mean arterial pressure and vasodilation that was followed by a decrease in diastolic pressure later at the steady state phase. They concluded that vasodilation observed after induction could be associated with the effect of propofol that disappeared with no significant change in hemodynamic parameters over time ²⁹. Another study within clinical parameters of anesthetic use in neurosurgical procedures reported that CVR remains constant due to the balance between vasoconstrictive and vasodilatory properties of intravenous and inhaled anesthetics ¹³. Additionally, among other volatile anesthetic agents in humans, sevoflurane has been shown to have the least vasodilatory effect; while its vasodilatory effect is more prominent on non-humans ^{1,6,30-32}.

In the present study, BOLD-CVR mapping showed increased BOLD-CVR values under anesthetic in the cerebellum, anterior cingulate, right temporal gyrus, and left frontal gyrus. The increase in BOLD-CVR under anesthesia could be explained by the confounding effect of anesthetics over hypercapnia

challenge. Given the timing of administration of the anesthetic agents, we interpret that the volatile anesthetic agent played the dominant role in cerebral vasodilation rather than propofol that resulted in higher cerebrovascular reactivity response compared to awake state. In addition, we conclude that even though volatile anesthetics such as sevoflurane have a partially intrinsic vasodilatory effect, their impact on cerebral vasculature is strengthened during BH challenge and hypercapnia. Further studies are needed to explicate the nature of this pattern.

Meanwhile, it is notable that a few clusters located in the occipital lobe demonstrated decreased BOLD-CVR in anesthetized state. The significant group difference in BOLD-CVR involving visual-related brain regions of the occipital lobe and lingual gyrus could be driven by higher visual system activity in awake state during breath holding. Activation of the visual system has been reported in other studies because of visual cues during performing breath-holding task^{33,34}. Also, our observation is in accordance with a previous study reporting that the occipital lobe may function in a opposite direction from the responses in the frontal, parietal, and temporal lobes in anesthetized state with sevoflurane³⁵.

-Effects of Anesthesia on Neuronal Activity

It has been reported that intravenous and inhaled anesthetics have similar impact on neuronal activity in a dose-dependent manner^{4,31}. From physiological perspective, they act on the ion-channel proteins that are anesthetic-sensitive and involved in neuronal excitability. The spread of neuronal excitation mostly in supra and infra granular layers of cortex has been shown to be dependent on γ -aminobutyric acid A (GABA_A), which is the main fast inhibitory neurotransmitter receptor in the central nervous system. Volatile anesthetics modulate GABA_A receptor function enhancing the inhibitory mechanism in synaptic and extrasynaptic receptors. As such, GABA_A-mediated inhibition may manifest as a global reduction of BOLD response driven by a reduction of neuronal excitation through thalamic input and intra-cortical processing³⁶⁻⁴¹. Additionally, volatile anesthetics such as sevoflurane enhance the inhibitory effect of glycine receptors involved in inhibitory neurotransmission in the central neuronal system⁴². Such effects have been demonstrated in an animal study, in which several anesthetics including inhaled isoflurane induced a decreased BOLD response associated with the suppression of neuronal activation⁶.

In the current study our results revealed that anesthetic agents caused a reduction in the activation area and magnitude of BOLD signal. Taken together, through the comparison between wakefulness and anesthesia states we speculate that inhaled anesthetics may complicate neurovascular coupling in which neuronal and vascular contributions of the hemodynamic response work in different ways. Therefore, it appears that these agents have a greater impact on neuronal activity (BOLD response) than on vascular effect (BOLD-CVR response). Hence, in all the studies including general anesthetics the key point to interpret the outcomes is to distinguish between neuronal excitability and vascular effects associated with using drugs.

-Effect of Anesthesia on Signal Variability

In addition to the above-mentioned results, our study highlighted the effect of general anesthesia on variability of BOLD and BOLD-CVR responses. From voxel-wise Levene's tests we have found that spatial variability of BOLD and BOLD-CVR responses decreased in anesthetized compared to awake state. Our observations agree well with the previous literature comparing the variability of BOLD response in awake and anesthetized states⁴³. Specifically, it has been proposed that anesthetic agents reduce the overall dynamic complexity or neuronal variability that is primarily driven by physiological and motion noises⁴⁴. With regards to the quantification of CVR, Bright et al. 2013 showed that implementation of BH fMRI task in clinical studies could be challenging due to the highly variable performance of BH challenge in awake state¹⁸. However, our study proposed lower variability of BOLD-CVR responses due to the control over the physiological factors and motion artifacts that were minimized under anesthesia. We suggest that implementation of BH fMRI studies under general anesthesia improves the robustness with less variability of BOLD-CVR response, which can be utilized as a biomarker in study of patients with cerebrovascular diseases³⁷.

There are several experimental limitations involved in this study which need to be considered. In interpretation of the results, the underlying pathophysiology of epilepsy in our cohort must be taken into account since it could interact with effect of the anesthetics and bias the results. Epilepsy is a cerebrovascular disease that can disrupt the balance between excitatory and inhibitory drive at the synaptic level, leading to seizures. Alteration in neuronal circuits, including anterior nucleus of the thalamus (ANT), fronto-motor cortex, and fronto-temporal cortex is involved in the pathophysiology of epilepsy⁴⁵⁻⁵⁰. At the same time, since each subject served as their own control, their comorbid epilepsy is less likely to have been a major factor. Also, given the small sample size, this study should be considered as an explorative work. Further research should be conducted with a greater number of participants. Future studies are needed to examine the effect of general anesthetics on BOLD responses in other group of patients with cerebrovascular diseases, as well as on healthy controls. However, the latter would be challenging to carry out since it is unethical to do such clinical practice among healthy volunteer participants.

Finally, another factor is the type and dose of the anesthetic agents. In this study we used the standardized combination of propofol and sevoflurane in induction and maintenance levels, and as such, the generalizability of our findings is limited to these agents. Different types and dosage of anesthetics need to be examined in future studies to obtain a better understanding of the effect of anesthesia in cerebrovascular and hemodynamic responses in human subjects.

In sum, our results highlight the independent effects of general anesthesia on cerebrovascular reactivity and neuronal activity. We revealed that sevoflurane, an inhaled volatile anesthetic, may affect neuronal factors more significantly than cerebrovascular factors. While general anesthesia may help reducing variabilities arisen by physiological and motion artifacts, future studies need to consider the significant effect of anesthetic agents on neuronal activity that may impede a fair comparison between awake and anesthesia states, if longitudinal studies are to be done.

Methods

-Participants

A total of 9 patients (4 males and 5 females, age 28-60 years old) diagnosed with drug-resistant epilepsy (DRE) were recruited from the Comprehensive Epilepsy Center at Thomas Jefferson University Hospitals. All patients had a diagnosis of mesial temporal lobe epilepsy (mTLE) with unilateral mesial temporal sclerosis (MTS) according to standard clinical criteria. As part of clinical care for mTLE patients with MTS, laser ablation of amygdala-hippocampal complex was administered. In order to deal with a typically complex patient cohort and often heterogeneous in seizure characteristics and clinical history, we had restricted the cohort through stringent inclusion/exclusion criteria: history of drug-resistant mTLE; on stable AEDs and compliant with medication use; an average of at least 1 complex partial or secondarily generalized seizure compatible with mTLE per month; seizure symptoms and/or auras compatible with mTLE; video EEG showed evidence of seizures from one temporal lobe consistent with mTLE; MRI had evidence consistent with mesial temporal lobe sclerosis. Participants were selected for this study if they were appropriate candidates for laser interstitial thermal therapy (LITT) for the treatment of their DRE. This clinical scenario required patients to be scanned in preoperative scanning in an awake state and under anesthesia during the LITT procedure⁵¹. There was a two-week interval between the two scanning sessions; and each session consisted of two different sets of BH fMRI acquisitions. Informed consent was obtained from all the patients and their legal guardian(s). The study was approved by the institutional review board (IRB) of Thomas Jefferson University Hospital. All methods were performed in accordance with the relevant IRB-approved guidelines and regulations.

-Anesthesia administration

The second MRI scan was acquired during the LITT procedure and under general anesthesia. All patients were evaluated by an anesthesiologist and underwent institutional standard of pre-anesthetic preparation. Every patient received a standard induction of intravenous propofol (130-300 mg) 15-20 minutes before scanning. After endotracheal intubation, sevoflurane anesthesia was administered through the endotracheal tube and maintained with 0.6-1.2 mean alveolar concentration (MAC) and 100% Fractional Inspiratory Oxygen (FIO₂) during MRI acquisition. Mean arterial blood pressure was also maintained at 65-75 mmHg and end tidal carbone dioxide (ETCO₂) at 30-35 mmHg throughout the procedure. After MRI acquisition and the LITT procedure were completed, patients were reversed with Sugamadex 2-4 mg/kg, extubated as per routine, and were observed in a post-anesthetic care unit during their recovery.

-Data acquisition

Both MRI sessions were performed on a 3.0T Achieva Phillips scanner with an eight-channel head coil. fMRI images were acquired axially using a single-shot echo planar imaging (EPI) sequence in the same anatomical location prescribed for T1-weighted images. The T1-weighted imaging parameters used were: FOV=24.0 cm, voxel size=1.0×1.0×1.0 mm³, matrix size=352×352, TR=7.5 ms, TE=3.4 ms and slice

thickness=1mm. Functional MR imaging parameters were FOV=23.0 cm, voxel size=3.5×3.5×3.5 mm³, matrix size= 128×128, TR=2 s, TE=25 ms and number of averages=1.

-BH paradigms

The breath holding (BH) protocol consisted of two separate BOLD fMRI acquisitions with a 15-minute interval between scanning sessions. The BH task consisted of 40 seconds self-paced normal breathing followed by 20 seconds breath holding. This cycle repeated 5 times, and additional 40 seconds normal breathing incorporated at the end of each task for a total duration of 5 minutes and 40 seconds (Figure 1). This BH fMRI task was determined according to the designs suggested by the previous studies. For instance, Dlamini et al. 2018 reported that the minimum of 3 sec is enough to produce a detectable fMRI BOLD response. However, a longer period of BH (19.6-20.3 seconds) is required to produce a robust and reproducible BOLD response that could assure sufficient magnitude and number of voxels manifesting the response²⁰. In addition, J.J. Pillai et al. 2015 showed that 20-30 seconds of BH could provide consistent results with less interscan variability response that is feasible for healthy participants. As such, 20 seconds BH was selected in the cycles since this could be easier for the subjects to follow the task than longer durations. In addition, BH was performed with an end-inspiration to increase subject's comfort⁵².

-Clinical measurements

Hemoglobin concentration (Hgb) was obtained via a blood draw immediately prior to scan acquisition. Other clinical parameters were recorded during the scanning, including non-invasive blood pressure, peripheral capillary oxygen saturation (SpO₂), and end-tidal carbon dioxide (etCO₂). During the awake session, a nasal cannula was used to collect expired gases after exhalation to measure etCO₂; while during the session under general anesthesia, this measurement was performed by the ventilator through the endotracheal tube.

-Data analysis Functional MRI data were analyzed using AFNI (Analysis of Functional NeuroImages, (<https://afni.nimh.nih.gov/afni>)) software⁵³. The modules *afni_proc.py* and *3dDeconvolve* were used for preprocessing and fitting the respiration response function (RRF) to the BH BOLD signal. Following standard initial preprocessing, *3dtcat* in AFNI was used to remove the first 2 volumes of fMRI data in order to ensure the MR signal had reached a steady state. Slice timing correction was then applied. The next steps included motion correction by 6 rigid-body-movement parameters as covariates, removal of linear drift, and smoothing with a 4mm full-width half-maximum (FWHM) Gaussian kernel. All EPI volumes aligned with the low-motion volume in the dataset (min_outlier) using *3dvolreg* in AFNI. They were then concatenated and warped to standard Talairach space. The functional data were registered to the structural MPRAGE image using an Affine transform with 12 degrees of freedom, that was then normalized to the Talairach standard space. In addition, *regress_censor_motion_0.3* and *regress_motion_per_run* were employed to censoring of large motion (≥ 0.3 ~mm between successive time points, based on the motion parameters) as well as to regress out of outlier time points and motion

parameters to be modeled separately per run⁵⁴. The two trials of BH fMRI data within each session were concatenated, while keeping the awake and anesthesia scan sessions separate.

Further data processing was carried out across the region of interest (ROI) including gray matter and thalamus areas. Statistical Parametric Mapping 12 (SPM12) and *recon-all* in FreeSurfer were used to create gray matter and thalamus masks respectively. The threshold of 0.1 was selected to binarize the probabilistic maps. For each patient under LITT treatment, a mask of the implanted laser probe was made manually for each subject. Figure 2 illustrates one sample of the probe mask generated using structural data for one of the participants. With the aim of excluding the potential artifacts that arise from the probe, the area under the masks were removed from both structural data and statistical maps. This scenario was replicated on preoperative data in the awake state as well to make a fair comparison between the two states. All the 3D voxel-to-voxel arithmetic calculation were executed using *3dcalc* tools in AFNI.

-BOLD cerebrovascular reactivity (BOLD-CVR) estimation BOLD BH fMRI analysis

BOLD-CVR maps were estimated from each patient's BOLD signal time series for each voxel of the brain (gray matter and thalamus) with general linear model (GLM) using *3dDeconvolve* in AFNI. The respiration response function (RRF) proposed by Birn et al. 2008⁵⁵ was used to model the average BOLD signal induced by variations in respiration volume following BH task. This modeling is more complex than gamma variate function that is typically used to fit the hemodynamic responses in BOLD fMRI studies. Specifically, the RRF fits well with the respiration response where a single deep breath results in an early peak of signal that increase at 3 seconds, and then a later post undershoot reaches the signal magnitude peaks at 16 seconds. The response to the BH task can be modeled by a difference between two gamma variate functions as shown in the equation:

$$\text{RRF}(t) = 0.6 t^{2.1} e^{-t/1.6} - 0.0023 t^{3.54} e^{-t/4.25}$$

In order to evaluate both vascular and neuronal effect of anesthesia, BOLD activation maps were also obtained by employing the typical gamma variate function to model BOLD response⁵⁵⁻⁵⁷. Magnitude of BOLD-CVR and BOLD responses were extracted over the ROI mask (gray matter and thalamus) from regression coefficients (beta weight values) maps that correspond with the approximate signal change associated with hypercapnia challenges.

-Statistical analysis

Second-level group analysis was conducted to estimate the contrast between two states of awake and anesthesia. The maps of regression coefficients (beta values) over ROI mask between the two states were compared using the voxel-wise student t-test with *3dttest++* (the program of the AFNI package). Two-sided significant thresholding was chosen at p-value < 0.05 (family-wise error correction). Also, the cluster-size with minimum number of voxels in each cluster defined at 150 voxels. The estimation was conducted by Montecarlo simulation performed with *3dClustSim* from the AFNI package, and nearest

neighbor (NN) level set at 1 (face-wise bordering; n=6 voxels) ⁵⁸⁻⁶⁰. The group analysis was performed for both BOLD and BOLD-CVR maps between groups toward investigating the effect of anesthesia on both neuronal and vascular factors. Then, three separate masks were generated from the clusters showed significant positive and negative BOLD-CVR differences between awake and under anesthesia states, as well as the brain regions within the ROI mask by subtracting the first two masks. These masks were used further to assess the overall mean BOLD signal in two states of awake and under anesthesia.

-Homoscedastic test

To investigate the effect of anesthesia on the variability of BOLD-CVR and BOLD responses, the Homoscedastic test (Levene's test) was applied among regression coefficient (beta value) maps of the two states of awake and under anesthesia. This statistical analysis assesses the homogeneity of variances for a variable in two or more groups. The test assumes the variances are equal unless the p-value of Levene's test is less than the predefined significant level (typically 0.05) ⁶¹.

Declarations

Authors' contributions

F.A. designed the study, processed the acquired data and wrote the paper, M.A. helped with statistics and data analysis, M.T. helped with data processing and analysis, V.R. supervised and managed clinical and surgical aspects of the project and edited the paper, F. B. M. advised on data analysis and manuscript writing and editing, C. W. was the principal study supervisor, supervised clinical and surgical aspects of the project and helped with manuscript writing and editing. All authors reviewed the manuscript.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figures

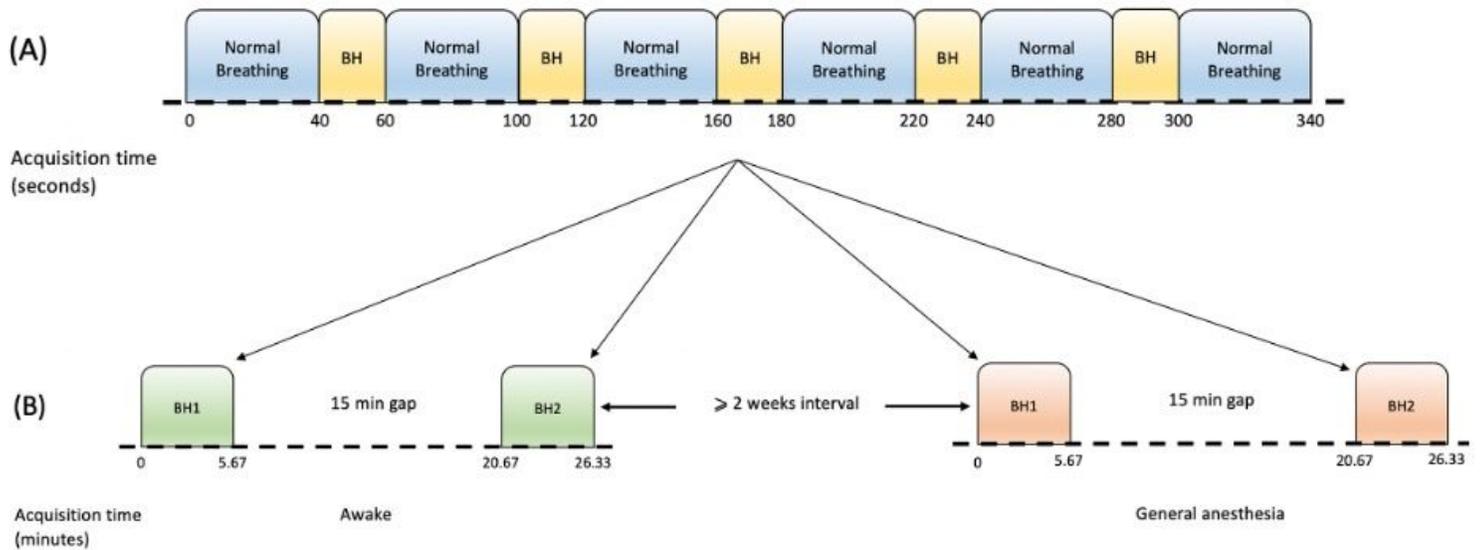


Figure 1

Schematic of BH paradigms. (A): each challenge was included 40 seconds normal breathing followed by 20 seconds breath holding and 40 seconds normal breathing added at the end of the task for recovery. (B): two times challenges applied at each session of awake and under general anesthesia with 15 min gap between each and at least a 2-week interval between scanning in awake and anesthesia states. During the second MRI session under general anesthesia, this BH paradigm was replicated by the anesthesiologist, who would manually control the ventilator.

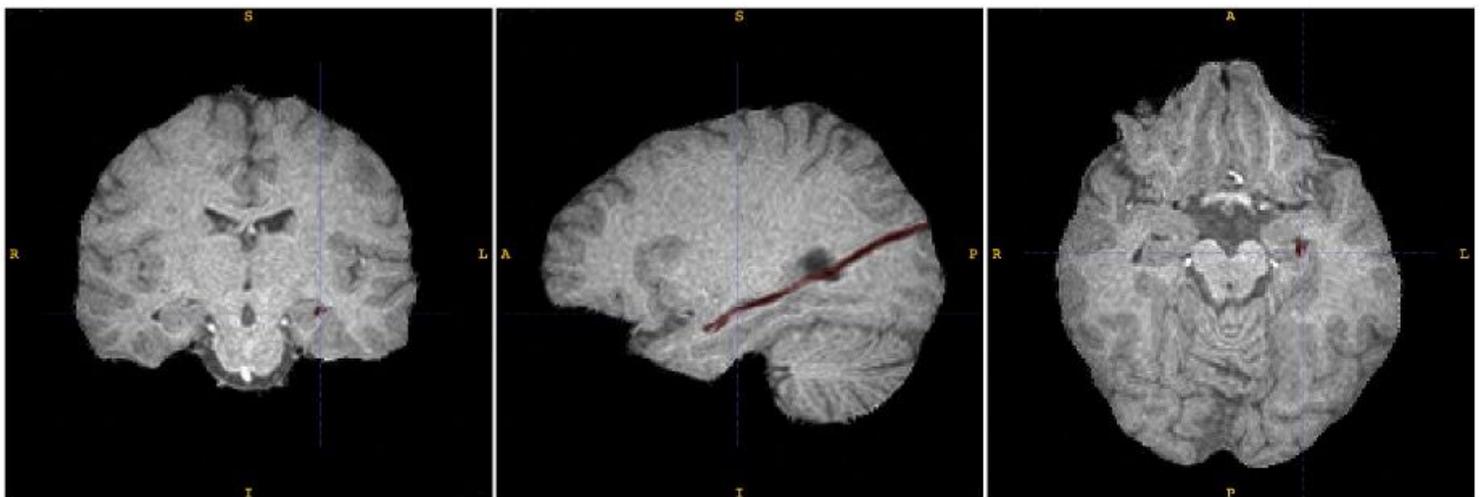


Figure 2

Sample mask of a probe created for one of the patients overlaid on the structural data.

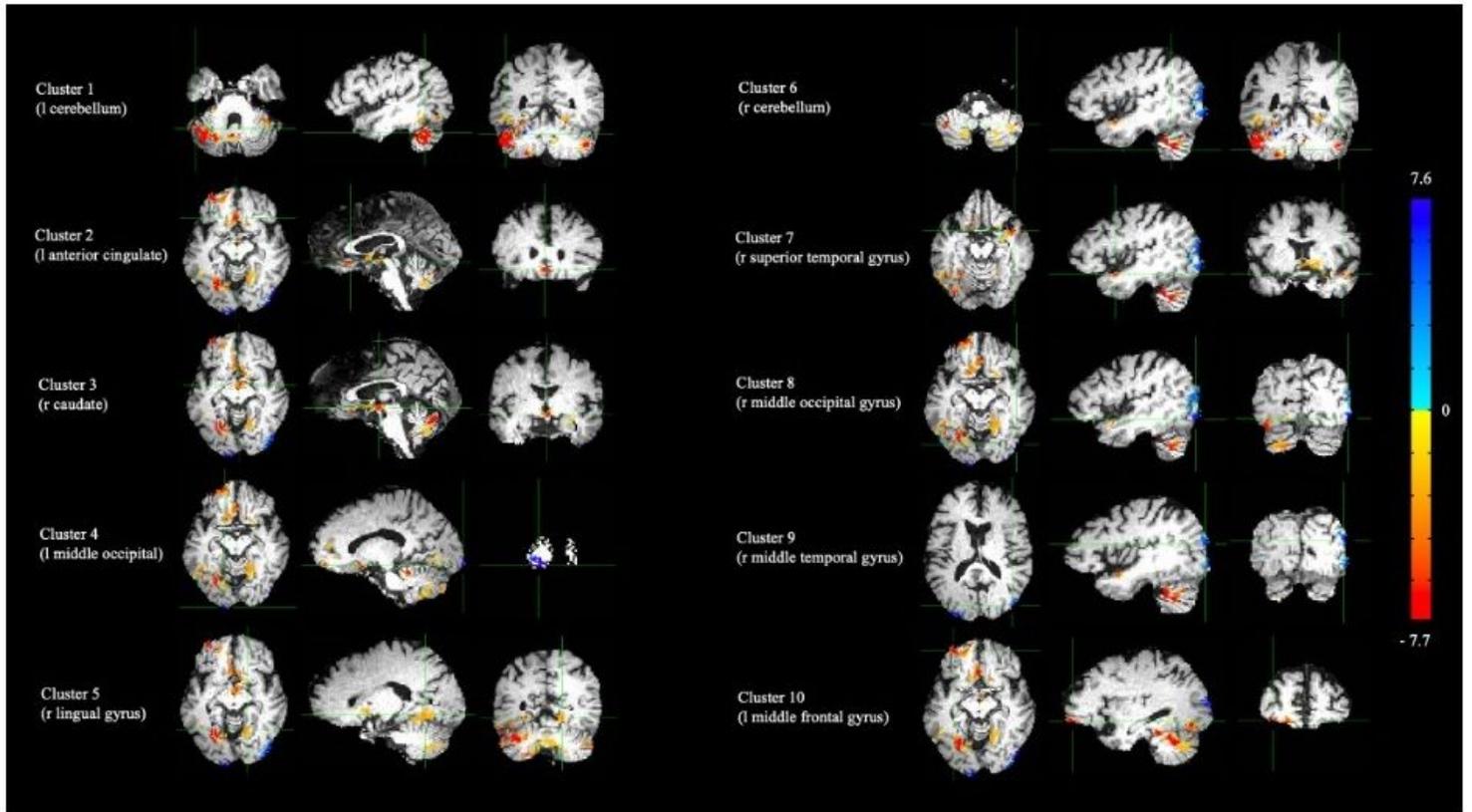


Figure 3

BH BOLD-CVR map of the group comparison between awake and under anesthesia states overlaid on MNI152 template shown at $p < 0.05$ with minimum cluster size of 150 voxels. Red corresponds with increased BOLD-CVR, and blue corresponds with decreased BOLD-CVR in anesthesia state).

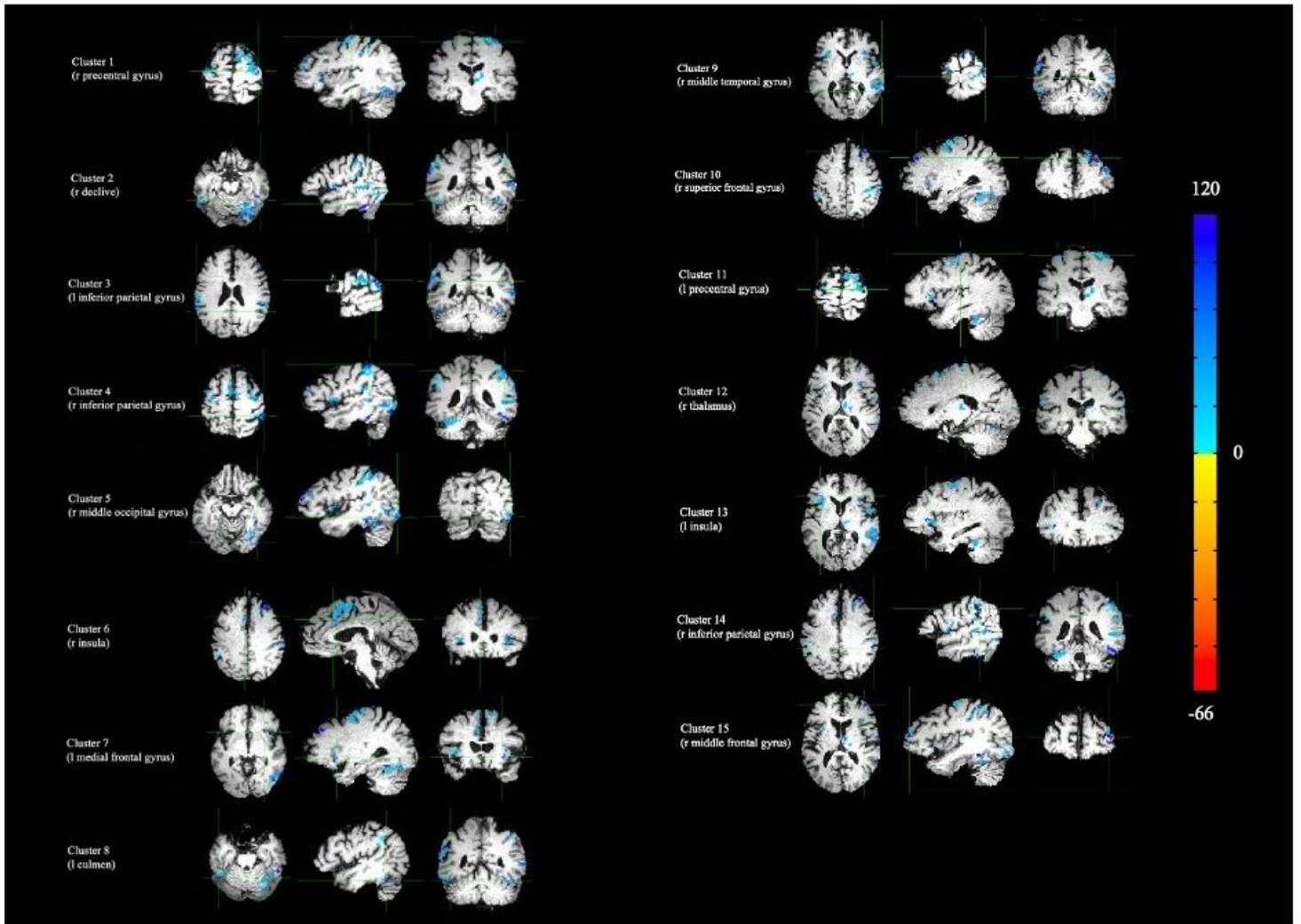
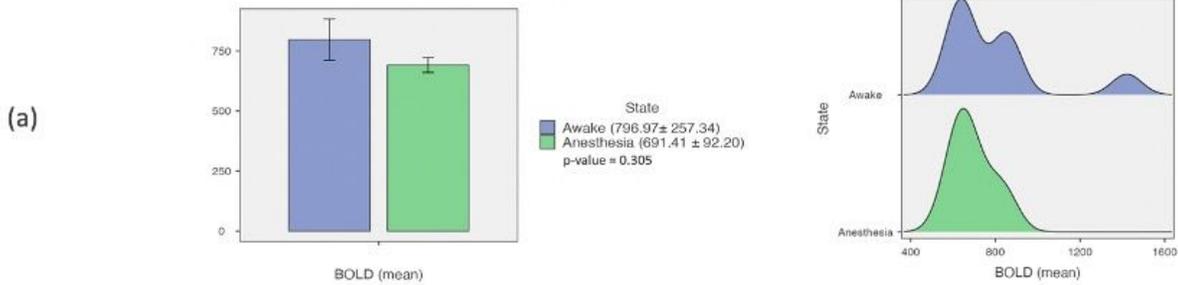


Figure 4

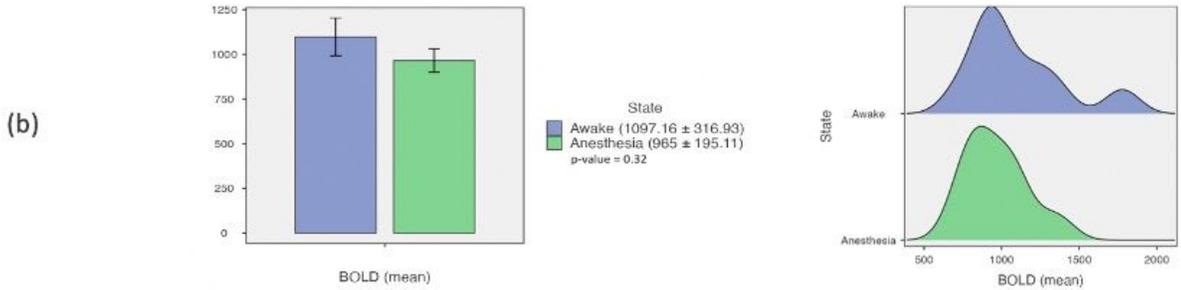
BH-BOLD map of the group comparison between awake and under anesthesia states overlaid on MNI152 template shown at $p < 0.05$ with minimum cluster size of 150 voxels.

Red corresponds with increased BOLD signal, and blue corresponds with decreased BOLD signal in anesthesia state ($p < 0.05$).

(Plots and density histograms of mean BOLD signal from the brain mask with significant **positive** BOLD-CVR differences between awake and under anesthesia states)



(Plots and density histograms of mean BOLD signal from the brain mask with significant **negative** BOLD-CVR differences between awake and under anesthesia states)



(Plots and density histograms of mean BOLD signal from the brain ROI mask by subtracting the masks of positive and negative BOLD-CVR differences between awake and under anesthesia states)

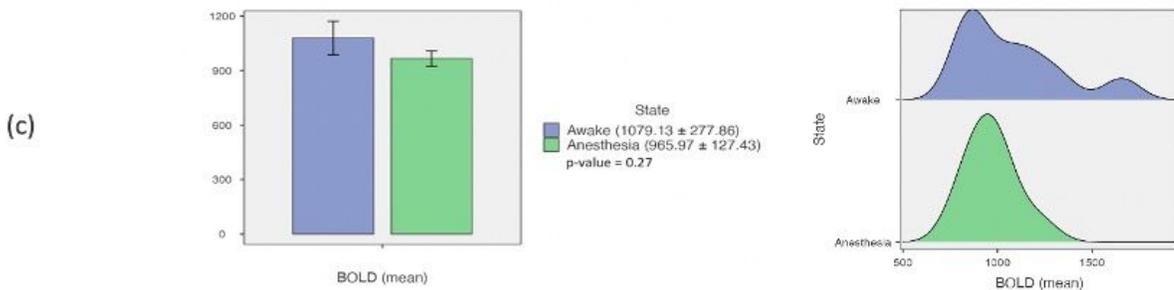


Figure 5

Plots and histograms of mean BOLD signal from the masks where the BOLD-CVR difference between awake and under anesthesia states was positive (a), negative (b) and the rest of the gray matter and thalamus mask (c) ($p < 0.05$). Each plot shows the mean and SD of the BOLD response extracted from the corresponding masks combined with related density histogram. The statistical t-test between awake and anesthesia states show non-significant differences between groups (t-test, p -value > 0.05).