Characterizing EEG Brain States During General Anesthesia in Children: Insights for Improved Brain Monitoring

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The role of my thesis advisors was invaluable: Emery N. Brown, MD, PhD: first conceived of the research concept; he wanted to explore and characterize the differences in pediatric versus adult EEGs under general anesthesia. He also provided research guidance and support. Patrick L. Purdon, PhD, my primary project mentor, helped me gain access to the clinical site (Massachusetts General Hospital) and to various hospital programs. He helped trouble-shoot major conceptual problems encountered during the project, and provided research and technical guidance and support. Analytic methods I employed in this paper were largely developed in the labs of Drs. Patrick Purdon and Emery Brown.

My role in this project was that of data collector (over half of the cases), project manager and initial trouble-shooter, data analyst, and manuscript author and editor. Aaron L. Sampson, B.S. oriented me to the lab when I first began the project and explained the in-house analytic tests relevant for my project. I collected 132 clinical cases for the project between January 2013 and September 2013 at Massachusetts General Hospital. I organized this data and extracted appropriate patient demographics and case-specific information for approximately 100 cases. I carried out all data quality assessment manually and ran the spectral analyses on the initial patient cohort. I wrote the primary spectral analysis codes used for the project, and carried out all initial statistical tests. After obtaining initial results and demonstrating proof of concept, Paul G. Firth and Kara J. Pavone helped collect an additional 26 patients to the final cohort. Karen Kan extracted relevant patient demographics and case variables for the final cases analyzed from the MGH Metavision database, and Kara J. Pavone, B.S. organized the case variables to determine clean periods of data for final analysis. Appropriate epochs for analysis were verified for over half the cases by myself and for all cases by KJP and Oluwaseun Johnson-Akeju, MD. Data analysis on the final set of cases was run by OJA. Initial manuscript drafting was completed by myself including the abstract, introduction, methods, and conclusion. This was critically edited by OJA as well as other members of the lab. OJA provided final results for the results section. The submitted manuscript and responses to the editor were critically edited by all members of the lab.
FUNDING AND DISCLOSURES

There is currently a patent pending for a closed loop anesthetic monitoring and delivery system based in part on this work and other data from the Purdon and Brown Labs. Preliminary data from this work were presented at the American Society of Anesthesiology 2013 Conference in San Francisco, CA, and to the Massachusetts Medical Society (MMS) executive board in December 2013. This work was awarded the MMS Information Technology Award in December 2013, and the Judah Folkman Prize for Clinical / Translational Science Research at the Soma Weiss Annual Research Day at Harvard Medical School in January 2014. This work was previously published in: Akeju O, Thum JA, Pavone KJ, Firth PG, Westover MB, Puglia M, Shank ES, Brown EN, Purdon PL. 2015. “Age-dependency of sevoflurane-induced electroencephalogram dynamics in children.” Br J Anaesth 115 Suppl 1:i66-i76.

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ABBREVIATIONS

ANOVA = analysis of variance
BIS = bispectral index
CNS = central nervous system
dB = decibels
DoAM = depth of anesthesia monitor
EEG = electroencephalogram
GA = general anesthesia
GABA = gamma-amino butyric acid
LOC = Loss of consciousness
MRI = magnetic resonance imaging
n = number of subjects
N₂O = nitrous oxide
PSI = patient state index
R² = coefficient of determination
RMSE = root-mean-square error
SAS = spectral anesthetic signature
TGTS = two group test spectrum
YA = young adult
ABSTRACT

Current brain monitors in clinical anesthesia use electroencephalography (EEG) based single indices (patient state indices, PSIs) as a biomarker for level of unconsciousness. These values range from zero (isoelectric) to one-hundred (fully awake and alert). However several variables contribute to the non-precise application of these PSIs to operative patient care. One important variable that obscures the interpretation of the PSIs for depth of anesthesia monitors (DoAMs) is patient age. DoAMs are used less frequently in children because operatively safe clinical PSIs do not reliably correlate with the patient’s clinical exam. Nonetheless, reliable monitoring of brain activity remains an important issue for managing anesthetic care given increasing concerns about adverse neuro-cognitive effects following general anesthesia (GA) in vulnerable populations, including pediatric patients. However, a detailed analysis of EEG signals has not yet been performed in children under GA, and reliable standards of care for brain monitoring during GA have yet to be established. In an effort to establish a robust biomarker for loss of consciousness (LOC) under GA regardless of patient age, I investigated how sevoflurane, the most commonly used pediatric GA, affects brain function in childhood and young adulthood.

Brain activity from patients aged 0 to 28 years (n = 54) was recorded using a 4-lead electroencephalogram (EEG) during routine care of patients receiving GA in this cross-sectional study. The EEG was characterized as a function of age and within 5 age groups: <1 yr old (n=4), 1–6 yr old (n=12), >6–14 yr old (n=14), >14–21 yr old (n=11), >21–28 yr old (n=13) which were determined based on developmental milestones and grouping after inspection of the data. The EEG power spectrum analysis using the multitaper method and coherence analysis were performed over a 10-minute period of stable anesthetic maintenance for each patient subgroup in 6 canonical EEG frequency bands.

Results showed that when compared with the adolescent (>14–21 yr) and young adult (YA) populations (>21–28 yr), EEG power in the pediatric population (>1 yr) was much larger, between 0.1–50 Hz, with a peak in total power around 5–6 years old. However for patients >1 yr, alpha band coherence structure was similar, and a distinct spectral signature consisting of a dominant alpha and slow oscillation emerged for patients of all ages, which is similar to the spectral signature seen in adult patients under sevoflurane GA. The only population this spectral signature did not apply to was infants (<1 yr), who did not exhibit prominent power or coherence in the alpha band, since neural circuitry necessary for this dynamic develops at about 1 yr of age.

The differences in EEG power and distribution of power across the canonical frequency bands at various ages helps explain why current DoAMs are inaccurate in kids. An ideal DoAM would be indicated for use in all age ranges. Since children >1yr and YAs have a reproducible, qualitatively similar EEG spectral signature under sevoflurane GA at operative levels, this may be used as a more reliable and robust biomarker for LOC. Furthermore, these similarities in the EEG spectra likely imply that similar underlying neurophysiological principles apply to the developing and developed brain to induce LOC. These EEG spectral anesthetic signature (SAS) patterns for various anesthetics have been associated with the onset of LOC in adults in behavioral studies in the Brown and Purdon labs, and may serve as an appropriate proxy for LOC in children as well. Based on these findings, a closed-loop brain activity monitor is being developed with the aim of dosing general anesthetics only until SAS patterns associated with the particular anesthetic drug in use is identified real-time. GA maintenance and emergence could similarly be moderated by the closed-loop system assuming the relationship between the
anesthetics used, and the SAS is known. This could potentially greatly decrease drug use and the neurotoxic effects of GA overdosing in the future.
INTRODUCTION

Overview

General anesthesia (GA) was introduced in 1846, and has since transformed the practice of surgery. There are approximately 40 million anesthetic procedures carried out yearly and 21 million patients a year received GA for surgical procedures in the United States alone as of 2007. Advances in monitoring, delivery systems, pharmacology, and standards of care have led to improved safety during GA. However clinical brain monitors have yet to improve standards of care in certain populations that are potentially at risk for GA induced neuro-toxicity. Current brain monitors in clinical anesthesia use electroencephalography (EEG) based indices (patient state indices, PSIs) that are graded on a scale of zero (isoelectric EEG) to one hundred (fully awake and alert). The frontal EEG used for these clinical devices is designed to be a surrogate for the drug effect on the entire central nervous system (CNS), though it is meant to measure the signal primarily from the frontal lobe which is critical to memory formation and recall. However depth of anesthesia monitors (DoAMs) are used less frequently in children because the PSI does not strongly or reliably correlate with pediatric patients’ level of unconsciousness, which is determined clinically.

At least three variables give rise to the non-standardized and potentially misleading clinical interpretation of these PSIs between patients: (1) general anesthetics used during the operation, anesthetic (2) dose-dependency of the EEG, and (3) patient age. Regarding the first variable, because different anesthetics act at different molecular targets in different neural circuits, the same index value does not assure the same level of unconsciousness for all anesthetics. For the second variable, the response of the EEG in each patient has also been shown to be dose dependent. In the transition from lighter anesthetic levels to deeper levels, the EEG under sevoflurane GA has been noted to change from dominant high frequency, low amplitude oscillations to alpha waves (8-13 Hz), to theta or delta waves, to burst suppression, and finally to an isoelectric EEG, with the authors of this dose-dependence study considering prominent alpha power to indicate adequate operative anesthetic level. This study, however, primarily addresses the characterization of how the EEG under sevoflurane GA changes with respect to the third variable: patient age. Prior to 1971, there had been a dearth of rigorous studies on the normal pediatric EEG, until 2013 when Eisermann et al. characterized the maturation of the EEG in various states of wakefulness using data primarily obtained from time domain EEG signals. These maturational stages of the EEG, summarized in figure 1, include an increase in the frequency of the background activity, as well as a discussion of the development of the occipital alpha wave secondary to the development of thalamocortical circuits at approximately four months to one year old. Changes in the awake pediatric EEG that are likely associated with normal brain development led us to hypothesize that potential differences in the pediatric EEG under GA compared to the young adult EEG would exist due to brain development as well.
Figure 1: EEG maturational stages depicting the developmental changes in the EEG during awake and asleep states between the ages of 0 and 20 years. The background EEG activity while awake showed an increasing trend from 3 Hz at 3 months of age to 10-11 Hz at 20 years of age. This could contribute to a stronger alpha oscillation in young adults that may be a natural aspect of development.

Despite these hurdles to discovering a robust, reliable biomarker for LOC or level of unconsciousness, clinically meaningful monitoring of brain activity is an important issue for managing anesthetic care, especially in children and elderly patients who still experience adverse neuro-cognitive effects following GA. Elderly patients also experience an increased risk of morbidity and mortality from GA. A detailed analysis of the EEG has not yet been performed in children under GA, and standards of care for brain monitoring have yet to be established. Thus, there is an urgent need for an improved understanding of brain physiology under GA, especially in the potentially vulnerable pediatric population. Since sevoflurane is the most commonly used general anesthetic, this was the maintenance general anesthetic agent investigated in this study. This study suggests why these single-index brain monitors are not appropriate for use in children, and supports a closed-loop GA delivery device based on a new, more robust method of clinical brain monitoring which is indicated for use in all patients >1 yr, including the pediatric population.

General anesthesia-related mortality and morbidity

General anesthesia induces a reversible loss of consciousness characterized by unconsciousness, akinesia, analgesia, and amnesia while maintaining stable vital physiologic function. One of the commonly feared complications of GA, however, is mortality. Based on a study involving ten academic medical centers, anesthesia-related mortality in the 1950s was estimated to be 64 deaths per 100,000 procedures, with people over the age of 75 years old being at the greatest risk (21 deaths per 100,000 procedures). It was also noticed that these mortality rates were markedly affected by the provider type, and presumably varied clinical practices. Many of these deaths were attributed to cardiovascular complications. Introduction of pulse oximetry and capnometry to the clinical practice of anesthesia reduced anesthesia-related mortality by the late 1980s. More robust physiologic monitoring reduced anesthesia-related mortality to approximately 1 in 100,000 in the early 2000s. This led to implementation of clinical standards in anesthesia that required constant monitoring of vital physiologic signals such as blood pressure, heart rate, temperature, inspired/expired gases, oxygen blood saturation
(pulse oximetry), and EEG. However, the increasing number and length of surgeries, the increasing number of invasive surgeries, and the increasing number of surgeries in risky patients with co-morbidities, has caused the rate of anesthesia-related mortality to rise again.

Contributing to the growth in anesthetic use is that the clinical practice of anesthesia continues to spread outside the operating room: anesthesia is also used for therapeutic and diagnostic purposes. Most surgical anesthesia procedures still occur in ambulatory care operating room settings. As of 2007 there were approximately 10.8 million ambulatory surgeries/year, and this number has continued to grow with increased surgical and diagnostic technology. In 1996, pediatric anesthesia used to represent approximately 12% of the caseload (as estimated by a survey in France), but this absolute number has grown with increased surgical demand in this population (as measured by the growing need for pediatric surgeons).

This has led to the increased use of GA in the pediatric population.

**General anesthesia: other risks**

Aside from GA-related mortality there are a wide variety of other GA-related complications. Over-sedation due to GA can potentially lead to excessive mechanical ventilation and associated lung injury, neuromuscular irregularities from prolonged immobilization, and neuro-apoptosis or neurotoxicity, a potentially severe complication for a developing brain.

Case and research reports have demonstrated the epileptogenic capability of several anesthetics during use, with potential long-term effects, although this is an active area of debate. Multiple GA exposures, especially at an early age, have been associated with long-term learning disabilities or delays. Even brief exposures of the less mature neonatal brain to anesthesia may have neurotoxic effects. While the neurotoxic effects of general anesthetics have not yet been directly tested on the human brain, multiple scientific studies have illustrated the deleterious effects of prolonged or increased exposure to general anesthetics in numerous animal models. These animal studies have implicated both gamma-amino butyric acid (GABA) agonists such as inhaled anesthetics, propofol, and benzodiazepine, and n-methyl-d-aspartate (NMDA) antagonists such as ketamine and nitrous oxide, in neurodegeneration (mainly apoptosis), abnormal synaptic development, and learning and behavioral deficits later in life after exposure to GA during critical brain developmental periods. Some authors claim these findings are not relatable to human development.

There are numerous variables that contribute to the potential inability to apply animal models of neurotoxicity to the clinical human experience, including patients’ frequency of exposure to GA, patient comorbidities, difference in biomarkers of injury, use of bioprotective strategies clinically, and appropriate correlations of animal model developmental age to human developmental age. Nonetheless, certain models estimate the equivalent age of critical brain development that can be clinically affected by GA in humans to be up to three years old.

In the pediatric population it may thus become increasingly important to properly define “elective” surgeries that can potentially be postponed to an older age in order to avoid vulnerable periods of development (i.e. synaptogenesis associated with neural plasticity and learning) that may be associated with an increased risk of GA neurotoxicity (demonstrated in rat models). Despite the growing amount of interest in this topic, as of 2016 research shows that there is no consistent agreement on the topic of how long certain operative procedures can be postponed, and how parents should be advised on the issue of neurotoxicity. Neurotoxic effects in the elderly population are also raising concern, as acute post-operative cognitive decline and dementia even 12 months after surgery can persist in the elderly population. In one
study, 75% patients over the age of 60 still had mild cognitive decline, and 11% had severe cognitive decline following surgery.\textsuperscript{60} This study, however, focuses on the effects of GA on pediatric patients, while a sister study in the Purdon and Brown lab focused on characterizing changes in the EEG under GA in the elderly population.\textsuperscript{61}

While the exact mechanism causing neural injury after anesthetic over-dose is unknown, one theory posits that inflammation is the underlying pathology. The tight physiologic link between the CNS and the immune system suggests that aberrations to the CNS, particularly the brain, through anesthesia can induce a local or systemic response that activates certain parts of the immune system, while suppressing others (such as killer T-cells) leading to inflammation and possible neuroimmunologic imbalance.\textsuperscript{62}

Complications associated with under-dosing general anesthetics are more apparent in the intensive care unit: auto-removal of monitors and other devices, movement and increased oxygen needs, ventilator asynchrony due to conscious control of breathing, and post-traumatic stress disorder from intraoperative awareness (20,000 - 40,000 cases/year in the U.S.)\textsuperscript{63} and, potentially, pain.

**Sevoflurane general anesthesia**

Sevoflurane was first used clinically in Japan in 1990, and received Food and Drug Administration (FDA) approval in 1995. It rapidly became the most popular inhaled general anesthetic for induction of pediatric patients worldwide\textsuperscript{64,65}, and has completely replaced halothane (another inhaled anesthetic) in many countries due to sevoflurane’s safer cardiovascular profile. Sevoflurane can also be safely used in adult populations. Because it is the most commonly used general anesthetic clinically in kids, sevoflurane is analyzed in this study, although relatively little is known about its mechanism of action.

**Mechanisms of anesthesia**

Standards of care for monitoring a patient’s state of consciousness or brain activity have yet to be established, likely because we are still largely unable to link the molecular mechanisms of anesthesia (which is still poorly defined for several general anesthetics) to higher cognitive function, and to specific EEG activity.

Like sleep, general anesthetics act on the CNS and alter thalamo-cortical signaling. However instead of acting only on specific sleep nuclei, GA works on specific channels that act at a variety of effect sites. These drugs modulate neurotransmission through various ligand-gated ion channels, but for some, their exact mechanism is unknown (including sevoflurane). More is known about intravenous anesthetics such as propofol, which potentiates the gamma-aminobutyric acid (GABA) receptor complexes throughout the brain (fig. 2), thus increasing global inhibition.\textsuperscript{66,67} This alters the excitation/inhibition activity balance, which usually sculpts developing neural networks based on experience. Thus, exposure to anesthetics during a critical period\textsuperscript{68} could alter physiologic neuronal pruning or induce pathologic cell death thus transiently or permanently altering the pediatric brain. This may explain why developing pediatric brains are more susceptible to the toxic effects of anesthetics than adults.
Figure 2: The mechanism of action of propofol, an intravenous general anesthetic. This method of global GABA inhibition may also be the underlying mechanism of action of the inhaled general anesthetic sevoflurane. 69

Pediatric anesthesia relies primarily on inhaled anesthetics, such as sevoflurane. 70 Although one other study (n=10) 71 published at the same time as this study also sought to characterize the sevoflurane-induced EEG, the mechanism of action of sevoflurane on a neuro-circuitry level remains unknown. However sevoflurane has been postulated to have a similar mechanism of action to the GABA-mediated global inhibition mechanism of action of propofol. This has been supported by similar systems neuroscience work in the Brown and Purdon lab that showed similar SAS and frontal alpha coherence in the anesthesia-induced unconscious state under both propofol and sevoflurane. This similarity in the global EEG response suggests a shared molecular and systems-level mechanism for LOC by both anesthetics. 72 Conversely, similar analytics methods have shown that dexmedetomidine and propofol have very different SASs, which is consistent with a known difference in mechanisms of action between the two drugs. This study, also from the Brown and Purdon lab, suggests that propofol enables a deeper state of unconsciousness by inducing large-amplitude slow oscillations, which produce prolonged states of neuronal silence. 73

Brain monitoring

It has been known for many decades that GA induces specific EEG patterns in the brain. 74,75 All general anesthetics affect the EEG, as does nitrous oxide (N₂O). N₂O was commonly considered indispensable in anesthesia as an adjunct which allowed lower amounts of anesthetic to be active for prolonged periods of time. N₂O has been used in clinical anesthesia for over 160 years, however modern agents such as sevoflurane have a low solubility 76 and so rapid changes in depth of anesthesia are possible without the use of N₂O. 77 Because N₂O is not necessary for anesthetic maintenance of pediatric patients, cases in this study did not include
N₂O as a maintenance drug. The confounding effect on the EEG spectrum (which was not taken into account in prior sevoflurane studies on pediatric EEGs) also validated the use of N₂O as an exclusion criterion for this study.

Existing brain monitors reduce the EEG patterns into a single number or index which is supposed to assess anesthetic depth of unconsciousness. The bispectral index (BIS) monitor (Aspect Medical Systems) is the most commonly used DoAM. The BIS determines the patient's level of consciousness through a proprietary algorithm that measures information including the dominant frequency band in the power spectrum, and various measures of fast and slow waves. This information is consolidated to a PSI that range from 100 (awake) to 0 (complete absence of brain activity). However, this single-index is highly non-specific, has failed to improve rates of awareness during GA, and is not approved for use in children, and detailed EEG analysis has yet to be performed in this population under GA. Most pediatric EEG studies are not carried out in the clinical setting of anesthesia, but rather usually in sleep, which is a markedly different physiologic state from anesthesia, as discussed briefly in the prior section. Studies of EEG response across a broad age cohort are also few in number.

Other biomarkers extracted from a combination of EEG and auditory evoked potentials to differentiate the degree of the anesthetic effect from LOC to burst suppression have been studied, however the clinical utility of these levels of unconsciousness past LOC has not been correlated with a difference in clinical patient outcomes. These methods are also more clinically complex, and do not follow widespread, common practice for anesthetic care. This project sought to disrupt clinical care as little as possible, and used equipment that the anesthesiologists were already familiar with.

The research labs of Drs. Purdon and Brown have recently discovered unique, highly structured oscillations in the EEG that arise abruptly at the onset of loss of consciousness (LOC) and persist with patient unconsciousness during GA. Since then, the lab has characterized these abrupt changes in brain states for a variety of general anesthetics, over a range of patient ages (this study included). These spectral anesthetic signature (SAS) patterns which are unique to each general anesthetic may serve as objective neurophysiological endpoints that can be used to guide drug administration. These concepts are currently being applied to patient care through development of a closed-loop anesthetic monitor with real-time power spectrum biofeedback that helps drive anesthetic dosing based on maintenance of the biomarkers of LOC (i.e. the SAS). At the time of publication, these EEG SASs had not yet been studied in pediatric patients. My project quantifies the EEG brain states induced by sevoflurane in pediatric patients, and characterizes the EEG dynamics as a function of age and neural development across a broad age cohort, which had not previously been explored.
METHODS

Data acquisition and patient population

Following a protocol approved by the Massachusetts General Hospital (MGH) Human Research Committee, 4-lead EEG data were recorded using the Sedline brain function monitor (Masimo Corporation, Irvine, California) during routine care of patients receiving GA. The standard Sedline Sedtrace electrode array records from electrodes located approximately at positions Fp1, Fp2, F7, and F8, with ground electrode at Fpz and reference electrode approximately 1 cm above Fpz. The data were strictly observational, with no intervention or influence upon the anesthetic care team. Sevoflurane dosing was decided by the anesthetic care team throughout the maintenance period for all patients, but was between approximately 1.9-3.0% during the analysis window period (table S1). 215 cases were collected intraoperatively between January 2013 and April 1, 2014 on patients 0 to 35 years old. Sixteen additional cases were obtained retrospectively from previously acquired data from 2012. Patients administered propofol, ketamine, dexmedetomidine, isoflurane, or other inhaled or intravenous general anesthetics were excluded from this study due to the known or likely confounding effects of these medications on EEG measurements, leaving 201 patients. Patients >28 years old were excluded, leaving 69 patients. N₂O, though not a typical stand-alone general anesthetic potentially has a confounding effect on the human EEG up to a period of approximately 5 – 7 minutes according to ongoing data analysis. Patients who were administered N₂O less than 10 minutes prior to the analysis window were excluded from this study. Patients were still included if N₂O was administered after the analysis window. This left 61 patients. Other exclusion criteria were: patients who were too small to be securely fitted with the EEG electrodes, patients who had mental disabilities, and patients who were undergoing a procedure that interfered with the electrode connections. The final cohort of 54 patients was deemed suitable for analysis.

Variable extraction

The following variables were extracted from internal MGH Metavision operating room records using patient-specific medical record numbers for the remaining 54 patients: gender, age, weight, type of procedure completed, surgery start time, surgery end time, general anesthetic start time, general anesthetic end time, sevoflurane vol % expired, minimum alveolar concentration (MAC), other drugs (analgesics, neuromuscular blockade) used during the procedure, operating room number, date of the procedure, and sevoflurane, N₂O, and oxygen flow rates throughout the procedure (table S1). EEG data set codes were mapped to each of the appropriate patient records through an excel file.

EEG processing

The raw EEG data were downloaded directly from the Sedline monitors in the hospital. The EEG data were recorded with a pre-amplifier bandwidth of 0.5–92 Hz, sampling rate of 250 Hz, with 16-bit, 29 nV resolution. Electrode impedance was <5 kΩ in each channel. The EEG data was converted from its native .phy format to .edf readable data using a converter script. The .edf EEG data was opened with the data viewer and editor Polyman. The raw EEG data were manually checked for quality in all 5 channels. Noisy or empty data sets were excluded from further analysis. The remaining data sets were modified in Polyman so that their headers (which contained information such as EEG recording start/end time, sampling frequency, voltage units,
system gain, and system offset) were reformatted for reading into Matlab (MathWorks, Natick, Massachusetts). The modified .edf data was imported to Matlab, where it was parsed into a structure containing the case’s data file (five rows of data for each of the channels that had a variable amount of columns to match the data length), and a structure containing all of the header information.

The EEG amplitude was adjusted for system gain and offset. The start times for the EEG data sets were extracted from the header file and mapped to the particular file’s name, along with a mapping to the patient’s age in Matlab. The difference between the surgery start time and the EEG start time was calculated to determine the offset between the two events (generally the EEG start time was prior to the surgery start time). The analysis window was determined by manually re-examining the raw data in Polyman for a stable period of ten minutes during the surgery (with the offset factored in) that exhibited low noise, and with no transition to burst suppression or emergence. All of these patients received only sevoflurane and oxygen for maintenance of GA during the ten minute analysis window, and at least 10 minutes prior to the analysis start time, and regional nerve block techniques were not used during the analyzed or preceding EEG epoch.

Spectral analysis

Although all channels were manually inspected for quality within the analysis window, four of the data channels (not including the middle ground channel) were transformed into a Laplacian average of the data using the equation:

\[ x_{tL} = x_t - \frac{1}{4} \sum_{j=1}^{5} x_j, \text{where } j \neq t \]

so that the contribution of each of the two right-sided channels and each of the two left-sided channels was equal (i.e. leads FP1, FP2, F7, and F8). The purpose is to average out any potential independent noise from each channel into a single, combined data set. From this time-series data, which indicated the mV of electrical activity of the brain over time, the EEG spectrum was calculated using the discrete-time Fourier transform implemented in the Chronux toolbox. The spectrum of a signal is the representation of a time-domain signal in terms of the contributions of a range of frequencies to the signal over time. This is illustrated by figure 3 below (courtesy of Patrick Purdon, PhD).
Figure 3: Flow of data processing from the raw data in μV over time to the spectrogram, or frequency representation of the data in decibels over time and across a specified range of frequencies. A) The raw brain wave signal (EEG) is measured over time. This is called the time series signal. B) The signal can be broken down into a high frequency component (blue, 8-12 Hz) and a low frequency component (green, 0.1-1Hz) which acts as an envelope (shapes) the high frequency signal. C) The contribution, or amplitude of each specific, single frequency can also be calculated for a specific point in time, which is represented as the spectrum. D) Repeating this frequency decomposition over time forms a 3-dimensional plot which represents a collection of these spectra over time (x axis). This 3-D plot is called a spectrogram. E) The power dimension of the 3-D spectrogram can be represented as a colorized scale indicating the amplitude, or contribution that a specific frequency (y axis) contributes to the signal at a given time (x-axis). By colorizing based on amplitude, we can represent the spectrogram in only two dimensions, and better understand the dominant frequencies comprising the signal in the frequency domain.

Multitaper Method The EEG spectrogram (i.e., the frequency distribution of the signal’s power over the ten minute analysis window) was estimated using the multitaper method (spectral resolution 3 Hz, sampling rate Fs = 250 Hz, time-bandwidth product TW = 3, window length T = 2 s with no overlap, number of tapers K = 5) calculated using 2 second non-overlapping, continuous time windows. The frequencies of interest were 0 – 50 Hz. This range of frequency analysis capture all of the 6 canonical EEG frequency bands (0.1-1 Hz slow waves, 1-4 Hz delta, 4-8 Hz theta, 8-12 Hz alpha, 12-25 Hz beta, and 25-50 Hz gamma). Total power for the signal was defined as the contributions from all of the frequencies between 0.1 and 50 Hz. The spectrum for each patient was calculated by taking the median value for each frequency within the ten minute analysis window of stable anesthetic maintenance to avoid bias from potential noise in the data. Average power within each of the spectral bands, and total power, was calculated from the patient’s spectrum (fig. S1). Due to an order of magnitude difference in EEG power between the pediatric and young adults patients, the power data was converted to the decibel (dB) scale using:

$$Power (dB) = 10 \log_{10} Power$$
in order to transform the data into a more normal distribution.

We also computed an age-varying spectrogram using an overlapping (0.5 yr) moving window spanning a 2 yr range from 0-28 yr (fig. S2A) and between 0–1.5 yr (fig. S2C) by computing the median spectrum across patients in 0.5 yr age bins to qualitatively illustrate the transition of the spectrogram with increasing age. We also computed group-level spectra and spectrograms for sevoflurane epochs using the median values for each frequency at each time point across all patients within each age group: <1 yr old (n=4), 1–6 yr old (n=12), >6–14 yr old (n=14), >14–21 yr old (n=11), and >21–28 yr old (n=13) (fig. S3). The median was used instead of the average to avoid bias from outliers or influencers. 95% confidence intervals were computed using multitaper-based jackknife techniques (table S2). 90

Coherence analysis

Coherence is essentially a frequency-dependent correlation coefficient, and indicates how synchronous signals in two different locations are at a given frequency. The coherence $C_{xy}(f)$ between two signals $x$ and $y$ is defined as:

$$C_{xy}(f) = \frac{|S_{xy}(f)|}{\sqrt{S_{xx}(f)S_{yy}(f)}}.$$  

$S_{xy}(f)$ is the cross-spectrum between signals $x(t)$ and $y(t)$. $S_{xx}(f)$ is the power spectrum of signal $x(t)$. $S_{yy}(f)$ is the power spectrum of signal $y(t)$. Just as the spectrogram is a time-varying version of the spectrum, the coherogram is a time-varying version of the coherence, estimated using consecutive windows of EEG data. The multitaper method described in the prior section was again implemented to construct the coherogram across the various groups, to construct an age-varying coherogram across the ages of 0–28 years, and to construct an age-varying coherogram across the ages of 0–1.5 years. 95% confidence intervals were again calculated using multitaper-based jackknife techniques.

Statistical analysis

The two-group test for spectra (TGTS) and two-group test for coherence (TGTC) as implemented by the Chronux toolbox (http://www.chronux.org), a jackknife-based method, was used to compare spectral and coherence estimates between the specified groups. 91 This method accounts for the underlying resolution of the spectral and coherence estimates, and considers differences to be significant if they are present for contiguous frequencies over a range greater than the spectral/coherence resolution $2W$. Specifically, for frequencies $f > 2W$, the null hypothesis was rejected only if the test statistic exceeded the significance threshold over a contiguous frequency range $\geq 2W$. For frequencies $0 \leq f \leq 2W$, to account for the limited spectral/coherence resolution at frequencies close to zero, the null hypothesis was rejected only if the test statistic exceeded the significance threshold over a contiguous frequency range from 0 to $\max(f,W) \leq 2W$. A significance threshold of $p < 0.001$ for group comparisons was used (table. S2).
RESULTS

Fifty-four patients were included in the final analysis between the ages of 4 months and 28 years old. Patient demographics for the six patient cohorts are summarized in table S1. A majority of patients within the infant and toddler groups (<1 yr, 1-6 yr), were male. The most common surgeries that patients underwent in these two groups were urologic surgeries and hernia repairs.

Qualitatively, the spectra and spectrograms retained the same structure with age > 1 yr with three representative cases shown in fig. S1B-D and fig. S1F-H, fig. S2A, fig. S3. These spectra illustrate two distinct peaks, showing a dominant slow wave/delta oscillation (0.1 – 4 Hz) and alpha oscillation (8 – 12 Hz). Figure S3 also shows a less dominant theta band (“theta fill-in”) on the pediatric spectrogram that is not as apparent after approximately 13-15 years. There is also a strong alpha coherence that is retained with age > 1 yr. (fig. S2B, fig. S4) At < 1 yr, there is neither a dominant alpha wave on the spectrogram (fig. S1E, S2C), and subsequently no alpha coherence (fig. S2D) for this cohort.

The total power of the EEG (1-50 Hz), however, changed drastically, over the scale of at least 10 dB, with age. The power increased from birth to 5-8 yr, then decreased rapidly to a plateau at approximately 18 – 22 yr (fig. S1I). Each canonical EEG band also exhibited similar trends. The cohorts for analysis were in part parsed out based on these power and coherence trends into the six cohorts.

Quantitatively, the TGTS for both power and coherence confirmed many of the qualitative findings above, but in addition indicated that there are slight differences in the distribution of power across age, despite the majority of the power being concentrated in the alpha and slow/delta bands (table S2). Specifically, more of the power was distributed in the slow/delta waves in infants (group 1) vs. groups 4 and 5, and the amount of alpha power was relatively greater in groups 2-5 than group 1. Notably, groups 2-5 had strong overlapping coherence in the alpha band as well. Groups 4 and 5 had increased coherence in the beta/gamma frequency range (table S2). The latter phenomenon may be explained by increased frequency of administering neuromuscular blocking agents, which are known to decrease frontal EMG and thus the power in the beta/gamma bands, in groups 4 and 5.
DISCUSSION

The clinical community requires that all procedures are maximally safe to avoid injuring patients. This has proven to be especially important in the field of anesthesia; as of 2009, the leading cause of cardiac arrest in kids was anesthetic overdose. Depth of anesthesia monitors (DoAM) indirectly measure the level of anesthesia delivered as it ranges between efficacy and toxicity. While current BIS and other brain monitors measure the effect of anesthetics on their primary target—the CNS—monitoring methods that are also effective in the pediatric population need to be implemented clinically.

The ideal DoAM would be simple to use, not be influenced by the subject’s age or gender, and would accurately reflect whether a patient is unconscious. Such a perioperative EEG monitor has eluded clinical researchers. Current BIS monitors are not approved for use in children, as they are normalized to adult EEG data. BIS studies have likewise largely been unable to reproducibly associate BIS indices or PSIs with depth of unconsciousness measures in a variety of single anesthetics and anesthetic combinations. EEG changes produced by single anesthetic drugs in younger and older children have previously been unavailable making the development of an ideal monitor indicated for use across all ages difficult. The results from this study directly address this lack of data in the pediatric population under a single, common, isolated anesthetic (sevoflurane).

This study shows that while the sevoflurane-induced EEG power changes significantly from childhood to young adulthood across all clinically-relevant frequency bands: 1. there is a peak in sevoflurane-induced EEG power at approximately 4-6 years with a continuous decrease in power thereafter, 2. Compared with the adolescent (>14–21 yr) and young adult populations (>21–28 yr), EEG power in the pediatric population (>1 yr) was much larger between 0.1–50 Hz. 3. Alpha band coherence structure was similar between pediatric (>1 yr), young adult, and adult populations. 4. The qualitative form of the spectra featuring peaks in the slow-delta and alpha bands is retained independent of age down to at least 1 yr. 5. Infants (<1 yr) did not exhibit prominent power or coherence in the alpha band, suggesting that the neural circuitry necessary for this dynamic, develops at about 1 yr of age.

These findings support two very important concepts. First, this work serves as a proof of concept as to why current DoAMs like the BIS do not work in pediatric patients. Second, this study serves as a proof of principle that EEG spectral anesthetic signatures (SAS) previously identified in adults are maintained for young adults and pediatric patients at least down to 1 year of age.

Why current brain monitors do not work

This study of the pediatric EEG under sevoflurane GA shows why simplifying EEG data to a single index that represents depth of anesthesia (using spectral analysis or complexity measures) fails to accurately reflect variations in underlying physiology in the pediatric population. Because DoAMs such as the BIS compute proprietary indices based on EEG power and ratios of EEG power, both of which are significantly different in children compared to young adults, the PSIs will not be appropriate indicators of unconsciousness in children.

Although this was a cross-sectional (as opposed to longitudinal) study, the findings suggest that the EEG, even under anesthesia, is affected by brain development (neurogenesis, apoptosis, myelination, axonal growth synaptogenesis, pruning, etc.). Synaptogenesis begins approximately post-birth and synaptic density increases to maximum levels by approximately 2
yr of age,\textsuperscript{97} with strengthening of excitatory synapses to about 5 years of age.\textsuperscript{98} These two processes then both decrease and plateau at around 18 years of age, with functional MRI studies suggesting that up to 50% of synapses are pruned by approximately age 15.\textsuperscript{99} The increase of the power up to age 5-6 yr and subsequent steep decrease in power up to age 20-22 yr likely are a reflection of these various stages of development. The greater variation in power among pediatric likely reflects the large variability in rate of brain development in the pediatric population. A longitudinal, case controlled study may give better insights into this hypothesis. Nonetheless, the variability in the pediatric data brackets a challenging problem. This finding suggest that particular attention needs to be given to kids in the transition range from adolescence to adulthood in order properly monitor brain activity and to dose drug delivery not necessarily based on weight and physical age, but on the response of their brain under anesthesia (or so called “brain age”).

Anesthesia-induced alpha oscillations may also serve as a sign of variable brain development, and are thought to occur through resonant cortical and thalamocortical networks. The relative strengthening of alpha and beta oscillations observed after 1 yr might therefore reflect maturation and increased synaptic efficiency in cortical and thalamocortical circuits. The relative sharpening of slow/delta oscillations observed in children may parallel sleep-related developmental changes promoting declarative memory formation and growth. Thus, these age-related changes in sevoflurane-induced EEG oscillations may reflect systems-level neuronal changes that occur during development. Furthermore, because of the current implications in sevoflurane in a GABAA-ergic circuits, the changes in the EEG, especially within the first year of life, may be related to the changing state of the chloride ion channels during this stage of development.

**SAS as a novel brain monitoring strategy in pediatric patients**

These results also provide important insights into how children could be monitored during GA in the future. Since children and adults have qualitatively similar sevoflurane-induced EEG spectra featuring peaks in the slow-delta and alpha bands (the sevoflurane SAS), it is likely that similar underlying neurophysiological principles apply.

The practical clinical approach for brain monitoring would entail a new paradigm: identification of EEG SAS patterns associated with different anesthetic drugs and titrate the anesthetic dose appropriately to avoid unnecessarily over-dosing the patient. Because SASs have been shown to be reliable markers of loss of consciousness (LOC)\textsuperscript{100} in adults, further studies would need to show that: 1. SASs exit in pediatric patients for other commonly used induction and maintenance anesthetics and 2. the same behavioral correlation between LOC and onset of the SAS is present in the pediatric population. This potentially more sensitive, more robust surrogate for unconsciousness under GA could greatly decrease the risk of over-dosing anesthetics if the moment of LOC is identified and simply maintained effectively versus at potentially toxic levels. Thus, this new monitor may be more effective at maintaining high standards of care\textsuperscript{101} in the pediatric population. And because the anesthesia-induced EEG signals were found to be so much larger in children, the problem of anesthetic brain monitoring may in fact be easier to measure in children versus young adults. The robust pediatric signal means smaller, more fitted electrodes can also be used for monitoring. Increasingly effective technology, such as an SAS method of monitoring, can play a huge role in controlling health care costs\textsuperscript{102} by decreasing drug usage, recovery time, operating room time, and costs due to potential mortality or morbidity from over-dosing.
Limitations

The major limitation of this study is that it is not a controlled trial in which all anesthetic and other drug doses were equally administered, since the clinical practice was not altered (table S1). No awake baseline EEG reading was measured in most cases due to the technical difficulty of reliably recording from most of the younger pediatric patients prior to induction. Baseline differences in EEG power with age reported in other studies are not as profound as the changes with age observed. Even Dustman who shows a logarithmic decreased in power across all bands (fig. 5) only suggests approximately a .4-.75 log difference in amplitude in the progression from 6 year old to 76 year old subjects, which would be an overall minor contribution to the changes seen in this study. While baseline data was not directly obtained, the primary focus of this study is the pediatric EEG under GA, and that it is also prone to the effects of neural development, even more significantly perhaps than during sleep or resting states, indicating that brain monitoring methods cannot use absolute values of power or even power ratios to determine a patient’s state of consciousness.

While the n for cohort is > 30 (as stated by the central limit theorem as being sufficient for the data to approximately approach a standard normal distribution), the increased spread of pediatric data compared to the young adult data may call for an increased n among a larger age distribution to fit the power to age with an accurate model. Increased data sets may reveal a more accurate polynomial model between power in dB and age. However, in follow up research in the elderly population, the decreasing trend in total power after childhood appears to remain. Obtaining an increased n may also help attain more female patients. Most of the data came from male patients, likely due to the type of surgeries from which the data was collected (table S1). This may have biased the age at which the major decline in power began, since it has been suggested that male brains develop later than female brains.

Potential sources of error in this study include differences in skull thickness or increased bone density with age, but this would not account for the order of magnitude change observed. Furthermore, the comparison of normalized spectra may artificially show a significantly increased alpha in young adults patients for two reasons:

1. Increased dopaminergic transmission may be associated with upper alpha (10–12 Hz) power so decreased dopaminergic transmission (i.e. with sevoflurane if it’s mechanism of action is similar to propofol) will lead to decreased alpha oscillations as seen in the pediatric population, who are often dosed to an anesthetic level about 2x higher than adults. Thus, this effect may simply be a reflection of the pharmacologic mechanism of action of sevoflurane. Even so, this alternative mechanism may not account for the large difference in power distribution in the alpha band.

2. Despite an overall decrease in EEG power with age, studies have shown a particular decrease in slow-wave oscillations with age during sleep. The normalized spectra may simply be reflecting this shift in power distribution, as opposed to an explicit increase in the alpha band. However, Whitford shows a curvilinear negative relationship between the log of age and EEG which does not support the orders of magnitude difference in baseline power shown in this study. Also, the selective decrease in frequency bands during sleep are unclear, with reported decreases in the entire 0 – 10 Hz frequency band or delta and theta frequency bands. In either case, sleep is an inherently different dynamic situation from sevoflurane GA and should not be assumed to have the same EEG dynamics.
Finally, one may posit that these profound differences in power are just a reflection of anesthetic management differences rather than of age. The relevant differences in clinical practices between children and young adults include not using paralytics in children, and using higher anesthetic doses in children (table S1). It is known that taking out the proprioceptive signal for arousal (as in adults where paralytics are used) allows for a lower anesthetic requirement to maintain muscle relaxation via the CNS. This would imply that adults have a lower requirement for anesthesia so their power should be absolutely higher in the high frequency bands especially all else equal, but it is not. This difference in clinical management may in fact obscure even greater significant differences between the pediatric and young adult cohorts, given equivalent weight-adjusted anesthetic doses.

**Future work**

As mentioned before, this field would benefit from longitudinal studies to map the changing dynamics in a single, normal individual to confirm these findings. A larger study may help decrease the effects of normal neurodevelopmental variability. EEG analysis of pediatric patients needs to be carried out under other common anesthetic drugs to determine if SASs are consistent in the pediatric population, and qualitatively similar to adults SASs. Coherence measurements during surgery can also be calculated to measure if global coherence in kids is equal to the 11 Hz global coherence in adults. This can contribute to our understanding of the underlying developmental changes of the brain as it affects EEG structure.

**Context of this work**

In general, it is very difficult to get an accurate measurement of the level of consciousness (or even to define what this truly is) of a patient under GA. Thus, regional anesthetics, especially in children, should be used when possible. When GAs need to be used in pediatric patients, this study reveals that anesthetics may act as a major stimulus, especially for the un-pruned, underdeveloped pediatric brain circuitry. GA is a state of massive change in the pharmacological milieu, and receptors are thrown out of their normal range. In kids these changes can be even more profound than what we have seen in adults: there is clearly a significantly different dynamic system in the kids when their CNS brain circuits are driven than in young adults. Regardless of skull or cortical thickness differences between young adult and pediatric patients, this dynamic system changes significantly an order of magnitude after approximately 5 yrs old, and this magnitude of change is a phenomenon not reported in resting or sleep states.

The constancy of the SAS for sevoflurane suggests a robust new closed-loop monitoring method, as discussed before. However the magnified differences in power under GA (compared to sleep or resting states) from childhood to adulthood serve as an amplified probe for “equivalent brain age” under GA. This has implications for amplifying gross systems response characterization of patients in pathologic states (i.e. disease-induced pathology such as Alzheimer’s or schizophrenia, or drug-induced pathology due to drug abuse) clinically. Thus, additional information about the patient’s “brain health” may potentially be gleaned during an operation. However, systematic studies to determine other aspects of such measurements will be needed.

Until such advances are made, the magnified effects of age on EEG power under GA may partially explain why it is much more difficult to push pediatric patients into burst suppression under anesthesia, and also explains why current single-index DoAM are unreliable in the pediatric population. Hopefully greater insights into SASs for various general anesthetics can
also lend insight into underlying similarities and differences in the pharmacologic mechanisms of anesthetic agents which have thus far eluded modern science.
CONCLUSION

Current single-index depth of anesthesia clinical brain monitors such as the BIS do not work in pediatric patients because the EEG power and ratios of power used to determine the patient state index are subject to significant variable, amplified effects of neural development with age. A new paradigm for brain monitoring during anesthesia must be introduced in this vulnerable population to avoid GA associated neurotoxicity, and intraoperative awareness. This study’s findings suggest that spectral anesthetic signatures (SAS) of the EEG would be a reliable means for monitoring patient loss of consciousness (LOC) since these SAS patterns are maintained with age, and thus indicated for use in children. Until researchers and clinicians are able to quantify consciousness, anesthesiologists will continue to face difficulties with accurately monitoring depth of anesthesia. However this robust novel technology may present a sufficiently sensitive method for brain monitoring, that is maintained across all ages and both genders.
SUMMARY

Neurotoxicity due to GA exposure is a growing concern in pediatric anesthesiology. However, current clinical EEG DoAMs are not appropriate for use in pediatric patients under GA. These EEG monitors do not work for monitoring children’s brain activity because the EEG changes with age under GA.

This study determined that the total EEG power changes as a function of age, and likely parallels changes in brain development. The difference in EEG power from birth to approximately 6 yrs is 10 dB. Similarly the EEG declines after this age > 10 dB. Thus the dynamic CNS circuits interacting with GA are significantly more active in children aged 5-8 yr old than in infants and young adults, even above normal differences in power seen at rest or during sleep.

Despite these changes in absolute power with age, SASs remain qualitatively constant with age under sevoflurane at least down to 1 yr old. Thalamocortical circuits necessary for the alpha rhythm for the sevoflurane SAS are not properly developed until approximately 1 yr old. The SAS for sevoflurane has dominant peaks in the slow and alpha bands from 1 yr to 35 years old. Varying amounts of theta fill-in can contribute to the SAS at younger ages, but the relevance of these oscillations is unknown. SASs have been shown to be associated with LOC in adult patients in prior studies. Further studies would need to determine if the onset and maintenance of SASs is associated with onset and continued LOC in pediatric patients, however this work is suggestive of this phenomenon.

Based on this work, a novel, more accurate and robust DoAM based on the SAS (which is potentially associated with LOC independent of age and gender) can be easily implemented in the operating room, and is based on simple pattern recognition by the clinician or by a computer. Real-time identification of onset and maintenance of LOC can decrease health care costs by decreasing drug use, patient OR time, recovery time, and post-anesthetic complications and death.
Note: All supplemental tables are from the final, published manuscript.

Table S1 – Summary characteristics of all patients who received sevoflurane GA (no. pts = 54). MAC, minimum alveolar concentration; SD, standard deviation.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>&lt;1 yr (N=4)</th>
<th>1-6 yrs (N=12)</th>
<th>&gt;6-14 yrs (N=14)</th>
<th>&gt;14-21 yrs (N=11)</th>
<th>&gt;21-28 yrs (N=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs), mean (range)</td>
<td>0.7 (0.4-1)</td>
<td>3.2 (1.2-5.8)</td>
<td>10.8 (6.1-13.7)</td>
<td>18.5 (14.8-20.7)</td>
<td>25.5 (22.6-27.7)</td>
</tr>
<tr>
<td>Sex (male), N (%)</td>
<td>4 (100)</td>
<td>10 (83)</td>
<td>6 (43)</td>
<td>6 (55)</td>
<td>6 (46)</td>
</tr>
<tr>
<td>Weight (kg), mean (SD)</td>
<td>9.0 (0.8)</td>
<td>14.9 (1.0)</td>
<td>39.4 (11.6)</td>
<td>77.9 (20.0)</td>
<td>78.4 (21.5)</td>
</tr>
<tr>
<td>Length of surgery (min), mean (SD)</td>
<td>36.5 (12.4)</td>
<td>67.4 (37.3)</td>
<td>54.4 (18.2)</td>
<td>93.3 (61.7)</td>
<td>89.3 (60.4)</td>
</tr>
<tr>
<td>Sevoflurane (vol% expired), mean (SD)</td>
<td>3.0 (0.6)</td>
<td>2.7 (0.5)</td>
<td>2.5 (0.4)</td>
<td>2.1 (0.3)</td>
<td>1.9 (0.5)</td>
</tr>
<tr>
<td>MAC</td>
<td>1.3 (0.3)</td>
<td>1.2 (0.2)</td>
<td>1.1 (0.2)</td>
<td>1.0 (0.1)</td>
<td>1.0 (0.2)</td>
</tr>
<tr>
<td>Propofol (mg), mean (SD)</td>
<td>25.7 (11.5)</td>
<td>35.6 (23.0)</td>
<td>122.0 (63.7)</td>
<td>200.0 (56.6)</td>
<td>212.5 (56.7)</td>
</tr>
<tr>
<td>Fentanyl (mcg), mean (SD)</td>
<td>15.0 (8.6)</td>
<td>20.8 (7.9)</td>
<td>62.9 (24.4)</td>
<td>155.0 (68.5)</td>
<td>152.1 (69.5)</td>
</tr>
<tr>
<td>Remifentanil (mcg)</td>
<td>30, N=1</td>
<td>12, N=3</td>
<td>0, N=0</td>
<td>0.75, N=1</td>
<td>0.8, N=6</td>
</tr>
<tr>
<td>Hydromorphone (mg), mean (SD)</td>
<td>N=0</td>
<td>N=0</td>
<td>N=0</td>
<td>0.9 (0.7), N=6</td>
<td>0.8 (0.6), N=6</td>
</tr>
<tr>
<td>Morphine (mg), mean (SD)</td>
<td>N=0</td>
<td>1.2 (0.8), N=3</td>
<td>2, N=1</td>
<td>3 (1), N=3</td>
<td>N=0</td>
</tr>
<tr>
<td>Ketorolac (mg), mean (SD)</td>
<td>N=0</td>
<td>9, N=1</td>
<td>18.8 (8.5), N=4</td>
<td>N=0</td>
<td>N=0</td>
</tr>
<tr>
<td>Neuromuscular blocking agents</td>
<td>N=0</td>
<td>N=3</td>
<td>N=4</td>
<td>N=9</td>
<td>N=10</td>
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**Table S2** – Summary statistics for analysis of power spectra and coherence between each group pairing.

<table>
<thead>
<tr>
<th>Group 1 (&lt;1 yr) vs Group 2 (&gt;1-6 yrs)</th>
<th>Power spectra (P&lt;0.0001, two group test spectra)</th>
<th>Coherence (P&lt;0.0001, two group test coherence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (&lt;1yr) vs Group 3 (&gt;6-14 yrs)</td>
<td>Group 2&gt;0.1-43 Hz; Group 3&gt;0.1-33.7 Hz;</td>
<td>Group 1&gt;0.1-2.9 Hz; Group 2&gt;4.4-12.2 Hz;</td>
</tr>
<tr>
<td>Group 1 (&lt;1 yr) vs Group 4 (&gt;14-21 yrs)</td>
<td>Group 1&gt;0.1-2.9 Hz; 22.5-50 Hz; Group 4&gt;3.9-19 Hz;</td>
<td>Group 1&gt;0.1-3.9 Hz; 13.7-50 Hz; Group 4&gt;8.3-12.7 Hz;</td>
</tr>
<tr>
<td>Group 2 (&gt;1-6 yrs) vs Group 3 (&gt;6-14 yrs)</td>
<td>Group 2&gt;27.9-34.2 Hz; Group 3&gt;1.5-9.3 Hz; 12.7-18.1 Hz</td>
<td>Group 2&gt;0.1-10.7 Hz; 13.7-50 Hz; Group 3&gt;0.1-4.4 Hz; 6.8-9.7 Hz; 13.1-38.6 Hz;</td>
</tr>
<tr>
<td>Group 2 (&gt;1-6 yrs) vs Group 4 (&gt;14-21 yrs)</td>
<td>Group 2&gt;0.1-50 Hz</td>
<td>Group 2&gt;0.1-10.7 Hz; 15.1-45.9 Hz; Group 4&gt;0.9-3.9 Hz; 15.6-34.2 Hz;</td>
</tr>
<tr>
<td>Group 3 (&gt;6-14 yrs) vs Group 4 (&gt;14-21 yrs)</td>
<td>Group 3&gt;0.1-50 Hz</td>
<td>Group 4&gt;0.1-45.4 Hz</td>
</tr>
</tbody>
</table>
FIGURES

Note: All supplemental figures are from the final, published manuscript.

Fig 1 Trends in spectra, spectrograms, and total power with age from 0 to 28 yr old. (a-d) Representative frontal EEG spectra illustrating that slow (0.1–1 Hz) and delta (1–4 Hz) oscillations are present in all patients during general anaesthesia, maintained solely with sevoflurane. Alpha (8–12 Hz) oscillations appear to emerge after 1 yr old. (e-h) Representative frontal EEG spectrograms illustrating that slow (0.1–1 Hz) and delta (1–4 Hz) oscillations are present in all patients during general anaesthesia. Alpha (8–12 Hz) oscillations appear to emerge after 1 yr of age. (i) Total EEG power (1–50 Hz) for each subject, plotted as a function of age. The total EEG power exhibited an increase from infancy, peaked at approximately 5–8 yr old, and subsequently declined with increasing age. The green line represents a fourth degree polynomial regression model describing the relationship between age and EEG power. The shaded bounds represent the 95% confidence bounds of this regression model.
Table S2

![Sevoflurane GA age spectrogram](image)

![Sevoflurane GA age coherogram](image)

![Sevoflurane GA age spectrogram](image)

![Sevoflurane GA age coherogram](image)

**Fig 2** Age varying spectrograms and coherograms during sevoflurane general anesthesia. (A) An age varying (1–28 yr) spectrogram representation of the EEG, showing that even though the EEG structure appears qualitatively preserved for all age ranges (presence of slow, delta, theta, and alpha oscillations), the power of these oscillations change as a function of age. (B) An age varying (1–28 yr) coherogram illustrating stably maintained, prominent alpha oscillation coherence. (C) An age varying spectrogram based representation of the EEG showing the relative absence of a well-defined alpha oscillation band at less than 1 yr of age. At approximately 1 yr of age the alpha oscillations become prominent. Slow, delta and theta oscillations are present from 0–1.5 yr. (D) An age varying coherogram illustrating the absence of alpha oscillation coherence, and slow delta band coherence, at less than 1 yr of age. This EEG dynamic appears at >1 yr of age, and is inversely correlated to slow oscillation coherence.

Figure S2
Figure S3

Fig. 3 Median spectra and spectrograms of age groups. (a, d) Group 1 (≤1 yr old). Both the power spectra and the group spectrogram show large power in the slow, delta, and theta frequency bands. A prominent alpha oscillation structure is noticeable absent. (b, e) Group 2 (2–6 yr old). Both the power spectra and the group spectrogram show large power in the slow, delta, theta, and alpha frequency bands. (c, f) Group 3 (6–14 yr old). Both the power spectra and the group spectrogram show large power in the slow, delta, theta, and alpha frequency bands. (g, i) Group 4 (14–21 yr old). Both the power spectra and the group spectrogram show large power in the slow, delta, theta, and alpha frequency bands. Compared with groups 2 and 3, the power in these bands is not as prominent. Power within the beta/gamma frequency band also shifts to lower levels. (h, j) Group 5 (21–28 yr old). Both the power spectra and the group spectrogram show large power in the slow, delta, theta, and alpha frequency bands. Compared with groups 2 and 3, the power in these bands is not as prominent. Power within the beta/gamma frequency band also shifts to lower levels.
Figure S4
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(Akeju, Jul 2015)