Prospective Study of Age-Dependent Changes in Propofol- and Sevoflurane-Induced Electroencephalogram Oscillations in Anesthetized Children

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ABSTRACT

General anesthetics induce structured brain oscillations that reflect activity in specific neural circuits and are readily visible in the electroencephalogram (EEG). In adults, frontal EEG patterns observed during propofol-induced unconsciousness consist of slow-delta oscillations (0.1-4 Hz) and frontally coherent alpha oscillations (8-13 Hz). Alpha oscillations are thought to reflect GABAergic mediation of thalamocortical dynamics, as evidenced by computational models, animal studies, and human studies. Given that the nervous system undergoes significant changes throughout childhood, it is not surprising that anesthesia-induced EEG oscillations in children have been found to differ significantly from those in adults. However, there has been limited work investigating the effects of general anesthesia on brain activity in children. The purpose of this thesis was to characterize the early changes in anesthesia-induced EEG oscillations in children and explore how these changes may reflect underlying neurodevelopmental changes. Specifically, this thesis describes work characterizing age-related changes in the EEG oscillations observed during (1) intravenous propofol general anesthesia and (2) volatile sevoflurane general anesthesia, two commonly used anesthetics in the pediatric population.

In Part 1, a prospective observational study was performed to investigate age-related changes in the propofol-induced EEG in children 0-21 years of age (n=97) using multitaper spectral and coherence methods. For patients 0-21 years old receiving propofol general anesthesia, total EEG power (0.1-40 Hz) peaked at about 8 years old and subsequently declined with increasing age. For patients greater than 1 year old, the propofol-induced EEG structure was qualitatively similar regardless of age, featuring slow-delta and frontally coherent alpha oscillations. For patients under 1 year of age, however, frontal alpha oscillations were not coherent. Given the distinct changes in the anesthesia-induced EEG observed within the first year, Part 2 of this thesis aimed to better characterize the EEG in very young children. In Part 2, a prospective observational study was performed to characterize the age-related changes in the sevoflurane-induced EEG in children 0-3 years of age (n=91) using multitaper spectral and coherence methods. For patients 0-3 years old receiving sevoflurane general anesthesia, slow-delta oscillations were present throughout all ages. Alpha oscillations emerged around 4 months old, anteriorized around 7 months old, and became frontally coherent around 10 months old.

These age-related changes in the general anesthesia-induced EEG likely reflect the development of the underlying neural circuits engaged by propofol and sevoflurane, possibly in inhibitory circuits and the thalamocortical connections implicated in propofol- and sevoflurane-induced unconsciousness. These anesthetic-specific and age-related signatures in the EEG
may also provide a unique opportunity to noninvasively study the development of neural circuits in humans. Furthermore, by understanding how the effects of general anesthesia change during development, we may be able to use EEG monitoring to develop more effective ways of tracking and establishing appropriate brain states in pediatric patients, and in doing so, enhance anesthetic safety.
TABLE OF CONTENTS

Abstract ........................................................................................................................................... iii

Acknowledgements ........................................................................................................................... vii

Statement of Research ....................................................................................................................... viii

Funding and Disclosures ..................................................................................................................... x

List of Abbreviations .......................................................................................................................... xi

Introduction .......................................................................................................................................... 1

Overview: General Anesthesia in Children .......................................................................................... 1

Possible Neurodevelopmental Effects of Early Anesthetic Exposure: Preclinical and Clinical Studies ............................................................................................................................ 1

Neural Circuit Dynamics Under General Anesthesia ........................................................................... 4

Monitoring Brain States Using the Electroencephalogram ................................................................. 5

Study Objectives .................................................................................................................................... 7

Part 1: Propofol-Induced EEG Oscillations in Children 0-21 Years of Age ......................................... 9

Methods .............................................................................................................................................. 9

Patient Selection ................................................................................................................................. 9

Data Collection ................................................................................................................................... 9

Spectral Analysis ................................................................................................................................. 9

Coherence Analysis ............................................................................................................................ 10

Statistical Analysis ............................................................................................................................. 11

Results ................................................................................................................................................ 13

Analysis of Patient Characteristics ................................................................................................... 13

Power Spectra Analysis ....................................................................................................................... 13

Coherence Analysis ............................................................................................................................ 13

Slow and Alpha Oscillations .............................................................................................................. 14

Infants Under 2 Years of Age ............................................................................................................ 14

Age-Varying Frontal Spectrogram and Coherogram ......................................................................... 15

Part 2: Sevoflurane-Induced EEG Oscillations in Children 0-3 Years of Age ................................. 16

Methods .............................................................................................................................................. 16

Patient Selection ................................................................................................................................. 16

Anesthetic Management ...................................................................................................................... 16

Data Collection .................................................................................................................................... 16
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I would also like to thank all members of the Neuroscience Statistics Research Laboratory (Massachusetts Institute of Technology, Massachusetts General Hospital) and the Berde Laboratory (Department of Anesthesiology, Critical Care and Pain Medicine at Boston Children’s Hospital) for welcoming me to the lab and for their advice and support over the past few years. In particular, I would like to thank Kristina Terzakis for her assistance with collecting data in children receiving propofol general anesthesia; Dr. Seong-Eun Kim, PhD for his guidance and generous assistance with modifying and implementing analysis methods; and Dr. Timothy T. Houle, PhD and Dr. Hao Deng, MB, BS for their statistical advice and assistance. Furthermore, I would like to sincerely thank Dr. Seun Akeju, MD, MMSc, and Kara J. Pavone, BA, for their selfless guidance, mentorship, and encouragement throughout my time in the laboratory. I also thank Dr. Paul G. Firth, MB, ChB, Dr. Erik S. Shank, MD, Elisa Walsh, MD, David Zhou, BS, and Dr. Michael Prerau, PhD, for their support and helpful discussions. I would also like to thank Sheri Leone for her administrative assistance.

This work would not have been possible without the gracious support of the hospital staff and subjects’ families. I would like to thank the pediatric anesthesiologists at Massachusetts General Hospital, the staff in the Pediatric Gastrointestinal Endoscopy Suite at Massachusetts General Hospital for Children, and the pediatric anesthesiologists, pediatric surgeons, pre-operative nursing staff, and operating room staff at Boston Children’s Hospital for their helpful assistance throughout these studies. I would like to thank Dr. Ann-Marie Bergin, MB, BCh, a pediatric neurologist at Boston Children’s Hospital, for reviewing all EEG recordings conducted at Boston Children’s Hospital for potential incidental findings in the subjects studied.

Finally, I would like to thank God for His steadfast love, grace, faithfulness, and provision, and my family and friends for their love, encouragement, and support throughout this journey.
STATEMENT OF RESEARCH

The work that is presented in this thesis represents original research that I carried out under the close guidance and supervision of my thesis advisors Dr. Patrick Purdon, Dr. Emery Brown, Dr. Laura Cornelissen, and Dr. Charles Berde. The role of my thesis advisors was fundamental to the completion of my research work: Drs. Brown and Purdon conceived the central concepts underlying my research work, namely the idea that general anesthesia produces brain oscillations that are readily visible in the electroencephalogram (EEG) and that the EEG patterns can be used to monitor brain states under general anesthesia. Dr. Brown provided valuable guidance and support, particularly for analysis and statistical methods. Dr. Purdon was my primary mentor and guided the direction of my project, provided guidance with data analysis methods, and provided advice and support throughout my work. The analysis methods I implement in this thesis were primarily developed in the Neuroscience Statistics Research Laboratory.

Drs. Brown, Purdon, Cornelissen, and Berde jointly developed the project investigating age-dependent changes in the EEG during sevoflurane general anesthesia in young children between the ages of 0 and 3 years. Dr. Cornelissen was my primary mentor at Boston Children’s Hospital. She mentored me throughout my research year from July 2015 to June 2016, and taught me how to collect and analyze multi-channel EEG data in young children. Drs. Cornelissen and Berde provided essential advice, support, and guidance throughout my research year.

This thesis work was adapted from the following publications:


My role in this project was that of primary author. With assistance, as detailed above, I collected EEG data from pediatric patients receiving propofol general anesthesia at Massachusetts General Hospital between June 2014 and August 2014. I also collected EEG data from children receiving sevoflurane general anesthesia at Boston Children’s Hospital between July 2015 and June 2016. The remainder of the data collected at Boston Children’s Hospital was collected by Dr. Cornelissen. I organized and reviewed the data for quality assessment, as well as extracted
information on patient demographics and clinical data. With guidance from those in the Neuroscience Statistics Research Laboratory, I wrote and ran the primary MATLAB analysis codes for the project. The final versions of the figures included this thesis are reproduced and/or adapted from the above publications by Lee et al. (2017) and Cornelissen et al. (2018) with permission. While I wrote MATLAB codes used to analyze data and generate preliminary figures under the guidance of Dr. Seong-Eun Kim, there were additional subjects recruited into the study after the completion of my research year, and the analyses and figures were modified accordingly.

In addition to the invaluable guidance and mentorship I received from my thesis advisors, I know that this thesis work could not have been completed without the assistance of the following people: Kristina Terzakis helped me collect data in children receiving propofol general anesthesia at Massachusetts General Hospital, with 155 clinical cases collected between Kristina and myself. Kara J. Pavone oriented me to the lab when I first joined the lab and introduced me to MATLAB and the data analysis methods. Dr. Seun Akeju guided me in modifying and implementing the MATLAB analysis codes for the data collected at Massachusetts General Hospital. Dr. Seong-Eun Kim guided and assisted me in modifying and implementing MATLAB analysis codes for the data collected at Boston Children’s Hospital.
FUNDING AND DISCLOSURES

This work was funded by the Harvard-Massachusetts Institute of Technology Health Sciences and Technology program; Massachusetts General Hospital Department of Anesthesia, Critical Care and Pain Medicine; and Harvard Medical School Scholars in Medicine Office.

Preliminary data from this work has been presented at the Massachusetts General Hospital Clinical Research Day on October 9, 2014, at Massachusetts General Hospital, Boston, Massachusetts; the Soma Weiss Student Research Day on January 15, 2015, at the Tosteson Medical Education Center, Harvard Medical School, Boston, Massachusetts; the International Anesthesia Research Society Annual Meeting on March 23, 2015, in Honolulu, Hawaii; and the National Student Research Forum on April 28, 2016, at the University of Texas Medical Branch, Galveston, Texas.

The work presented in this thesis has been published or will be published as peer-reviewed publications. The work investigating propofol-induced EEG oscillations in children was published in *Anesthesiology* in August 2017. The work investigating sevoflurane-induced EEG oscillations in children has been accepted for publication in the *British Journal of Anaesthesia* as of February 2018.

Conflicts of Interest: My thesis advisors and/or collaborators, Drs. Purdon, Brown, and Akeju, have submitted patent applications describing the use of electroencephalogram measures described in this article for monitoring sedation and general anesthesia. Some of these patents have been licensed to Masimo Corporation by Massachusetts General Hospital. Drs. Purdon, Brown, and Akeju are due to receive institutionally distributed royalties under this licensing agreement. Drs. Purdon and Brown had consulting agreements with Masimo Corporation in 2014 and 2015. Drs. Berde and Cornelissen have no conflicts of interests relevant to this work.
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<tr>
<td>aEEG</td>
<td>Amplitude-integrated electroencephalogram</td>
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<tr>
<td>AIMS</td>
<td>Anesthesia Information Management System</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BIS</td>
<td>Bispectral index</td>
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<tr>
<td>EEG</td>
<td>Electroencephalography, electroencephalogram</td>
</tr>
<tr>
<td>fNIRS</td>
<td>Functional near-infrared spectroscopy</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
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<td>GABA\textsubscript{A}</td>
<td>Gamma-aminobutyric acid type A</td>
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<td>GAS</td>
<td>General Anaesthesia versus Spinal anaesthesia</td>
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<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<td>NREM</td>
<td>Non-rapid eye movement</td>
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<td>PANDA</td>
<td>Pediatric Anesthesia Neurodevelopment Assessment</td>
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<td>SEF90</td>
<td>Spectral edge frequency 90%</td>
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INTRODUCTION

Overview: General Anesthesia in Children

In the United States, approximately 6 million children receive general anesthesia for surgical procedures each year (DeFrances & Hall 2007; Sun 2010; Rabbitts et al. 2010). General anesthesia is a drug-induced reversible state that is characterized by loss of consciousness, amnesia, analgesia, and immobility, accompanied by the maintenance of physiological stability (Brown et al. 2011). As a result, anesthesia is a necessary component of providing safe conditions for surgeries, procedures, imaging studies, and other diagnostic procedures in children (Sun 2010).

Possible Neurodevelopmental Effects of Early Anesthetic Exposure: Preclinical and Clinical Studies

Although general anesthesia is considered safe, animal studies have suggested that exposure to anesthetic drugs at a young age could have long-term neurodevelopmental effects (Sun 2010; Lin et al. 2014; Backeljauw et al. 2015; Andropoulos & Greene 2017). Specifically, several animal studies have found an association between early exposure to anesthetics and neuroapoptosis (Jevtovic-Todorovic & Olney 2008; Loepke et al. 2008). Many of the anesthetic drugs commonly used primarily act by (1) enhancing gamma-aminobutyric acid (GABA) receptors mediated inhibition (e.g. propofol, inhaled anesthetics), or (2) blocking N-methyl-D-aspartate (NMDA) glutamate receptors (e.g. ketamine) to decrease excitation (Jevtovic-Todorovic et al. 2003; Loepke et al. 2008). Both of these receptors play an important role in the development of the nervous system, and early anesthetic exposure during development could alter brain development and maturation by modifying GABA or NMDA-mediated neuronal activity (Loepke et al. 2008). The majority of animal studies have investigated the effects of ketamine (a NMDA antagonist) and isoflurane (an inhaled anesthetic that enhances GABA-mediated inhibition) on the developing brain. Early exposure to ketamine was found to induce apoptotic neurodegeneration in rats (Ikonomidou et al. 1999; Hayashi et al. 2002), mice (Young et al. 2005), and rhesus monkeys (Slikker et al. 2007). Furthermore, studies investigating isoflurane found that early exposure to isoflurane was associated with apoptosis in mice (Johnson et al. 2008), rats (Jevtovic-Todorovic et al. 2003), and rhesus monkeys (Brambrink et al. 2010; Zou et al. 2011). Propofol was also found to induce neuroapoptosis in mice (Cattano et al. 2008) and in rhesus monkeys (Creeley et al. 2013). This association with neuroapoptosis was also seen with ethanol (Ikonomidou et al. 2000; Olney et al. 2002; Young & Olney 2006), another GABA-mimetic agent. In addition to the apoptotic neurodegeneration observed
histologically, further studies investigating behavioral endpoints found that early exposure to anesthetics were associated with learning deficits in rats (Jevtovic-Todorovic et al. 2003; Stratmann et al. 2006).

How these studies conducted in animal models apply to human children receiving general anesthesia has been an area of ongoing investigation (Loepke et al. 2008; Jevtovic-Todorovic et al. 2008; Davidson 2011; Rappaport et al. 2015; Andropoulos & Greene 2017). While some retrospective studies have suggested a link between anesthetic exposure in early development and later neurocognitive deficits (Wilder et al. 2009; DiMaggio et al. 2011; Flick et al. 2011; Ing et al. 2012; Lin et al. 2014; Stratmann et al. 2014; Backeljauw et al. 2015), other studies have not supported this association (Bartels et al. 2009; Hansen et al. 2011; Hansen et al. 2013). Interpreting the results of this collection of epidemiological studies has been challenging because of many factors, including but not limited to difficulty distinguishing the effects of the underlying disease process or surgery itself versus the effects of general anesthesia, heterogeneous populations with a variety of anesthetic drugs and age groups, and heterogeneous neurodevelopmental outcome measures (Istaphanous & Loepke 2009; Stratmann et al. 2014).

Two recent large-scale prospective studies have suggested that there is no increased risk for adverse neurodevelopmental outcomes after single, short sevoflurane exposures (Davidson et al. 2016; Sun et al. 2016). The General Anaesthesia compared to Spinal anaesthesia (GAS) trial, an international randomized controlled trial, found no significant difference in neurodevelopmental outcome measured at 2 years of age between infants who received sevoflurane general anesthesia and infants who received awake-regional anesthesia before 60 weeks postmenstrual age (Davidson et al. 2016). Although neurodevelopmental testing at 2 years of age has limited sensitivity, results of the analysis of the primary outcome at 5 years of age will likely available in the near future (Davidson et al. 2016). The Pediatric Anaesthesia Neurodevelopment Assessment (PANDA) study prospectively studied neurodevelopmental outcomes in discordant sibling pairs, where one sibling had a single anesthetic exposure before 36 months and the other sibling did not (Sun et al. 2016). This sibling-matched cohort study found no significant difference in later cognitive function (assessed between 8 and 15 years of age) as measured by IQ (Sun et al. 2016). Both of these studies had relatively short anesthetic durations (median anesthesia duration 54 min (GAS), 80 min (PANDA)) for elective inguinal hernia surgery. While most anesthetic exposures in infants appear to be relatively short, there is still a significant proportion of infants who require longer durations of anesthesia (Davidson et al. 2016; Andropoulos & Greene 2017).
However, it is yet unclear whether repeated or prolonged exposures to anesthesia could have adverse effects on the developing brain. Some studies have found adverse effects with multiple early anesthetic exposures (Wilder et al. 2009; Flick et al. 2011; Hu et al. 2017) or prolonged anesthetic duration (Wilder et al. 2009). However, interpretation of these studies is limited, given that patients requiring multiple anesthetics or prolonged anesthesia could have congenital or chronic conditions that may increase risk for adverse neurodevelopmental outcomes (Davidson et al. 2016). There is also some evidence suggesting that anesthetic exposure at certain ages may be associated with adverse outcomes (Graham et al. 2016), suggesting there may be specific developmental windows at which the brain is sensitive to anesthetic exposure. Certain subgroups of children, including premature infants and infants with congenital heart disease or other comorbidities, may be particularly vulnerable to anesthetic exposure as well (Sun 2010). Further study is needed to better characterize the possible adverse effects of general anesthesia during infancy and childhood.

Taking these studies into consideration, the U.S. Food and Drug Administration (FDA) has issued a warning that prolonged exposure or multiple exposures to anesthetics have the potential to adversely affect brain development in children less than 3 years of age (FDA 2017). While many procedures requiring anesthesia cannot be postponed as they are medically necessary and could cause harm if delayed, the FDA and others have suggested delaying elective surgeries or procedures when possible and medically appropriate in children less than 3 years of age (FDA 2017; Rappaport et al. 2015; Andropoulos & Greene 2017). Nevertheless, there remains a need for better characterization of the possible adverse effects of general anesthesia and the relationship to the timing of anesthesia, type of anesthesia, and underlying medical conditions, among many other factors.

In the meantime, it is important to consider how to administer general anesthesia to children within this uncertain context. Typically, anesthetic drugs are dosed using population-based pharmacological models that account for a patient’s age, weight, and other variables (Struys et al. 2015). However, individual patients may respond differently to anesthetic drugs. In adults, the anesthetic concentrations required to induce unconsciousness can vary by as much as a factor of 2 above or below suggested doses (Iwakiri et al. 2005). Intraoperative awareness can occur if anesthetic drugs are underdosed (Palanca et al. 2009; Avidan et al. 2011). Moreover, there is growing evidence that exposure to anesthetic drugs in excess of what is required to maintain general anesthesia could have detrimental effects: children who receive greater than 4% sevoflurane can show epileptiform activity (Constant & Sabourdin 2012; Gibert et al. 2012), and adults who experience burst suppression – a state of anesthesia-induced coma
beyond what is required for unconsciousness – are at greater risk of post-operative delirium and cognitive deficits (Soehle et al. 2015; Fritz et al. 2016). Consequently, it is important to consider how to manage the level of anesthetic exposure when surgery under general anesthesia is required and cannot be postponed. One approach to managing the level of exposure would be to adjust anesthetic management such that anesthetic dosing is just sufficient to induce and maintain loss of consciousness. In order to do this, it is first important to consider the effects of general anesthesia on the brain.

**Neural Circuit Dynamics Under General Anesthesia**

General anesthesia has profound effects on the brain, inducing sedation and loss of consciousness by acting on various ion channels in the central nervous system (Hemmings et al. 2005; Brown et al. 2011). Propofol primarily acts on GABA type A (GABA<sub>α</sub>) receptors in the cortex, thalamus, medulla, brainstem, and other areas of the central nervous system, to enhance GABA<sub>α</sub> inhibition and thereby produce loss of consciousness, apnea, and atonia (Hemmings et al. 2005; Brown et al. 2011). Sevoflurane, an inhaled anesthetic commonly used in children, likely works via similar pathways but acts at many receptor targets, including GABA<sub>α</sub> receptors, NMDA receptors, glycine receptors, glutamate receptors, nicotinic acetylcholine receptors, and potassium channels (Campagna et al. 2003; Hemmings et al. 2005; Brown et al. 2011). The potentiation of GABAergic circuits by anesthetics is thought to produce the various features of anesthesia, including loss of consciousness, apnea, and atonia, by acting at specific neural circuits to cause specific behavioral or physiologic responses (Brown et al. 2011).

One of the main modalities used to investigate the neurological effects of general anesthetics has been electroencephalography (EEG). The EEG, which is measured at the scalp, reflects local field potentials generated by neuronal spiking activity in cortical and subcortical regions of the brain (Purdon, Sampson et al. 2015). Although the local field potentials generated by subcortical regions like the thalamus are much smaller in amplitude, cortical and subcortical regions are connected to and communicate with each other – as such, the observed EEG patterns reflect activity in both cortical and subcortical structures (Purdon, Sampson et al. 2015). Characteristic changes in the EEG with the administration of anesthetics, including pentobarbital and ether, have been described as early as 1937 (Gibbs et al. 1937). EEG patterns observed in adults during propofol-induced unconsciousness consist of large amplitude slow-delta oscillations (0.1-4 Hz) and frontally coherent alpha oscillations (8-13 Hz) (Feshchenko et al. 2004; Lewis et al. 2012; Purdon et al. 2013; Akeju et al. 2014; Purdon, Sampson et al. 2015). Similarly, sevoflurane in adults induces EEG oscillations consisting of
large amplitude slow-delta (0.1-4 Hz), frontally coherent alpha (8-13 Hz) oscillations, and frontally coherent theta (4-8 Hz) oscillations (Akeju et al. 2014; Akeju et al. 2016).

The characteristic slow and frontal alpha oscillations observed during both propofol- and sevoflurane-induced unconsciousness appear to relate to specific neural circuit dynamics. Slow-delta oscillations are thought to result from GABA-mediated inactivation of the arousal circuits (Brown et al. 2011; Lewis et al. 2012; Purdon, Sampson et al. 2015). Slow oscillations, which are present throughout the scalp, are thought to disrupt cortical integration by isolating local cortical networks and impairing both local and distant communication (Lewis et al. 2012). Slow-delta oscillations are also seen with many other anesthetics, including dexmedetomidine, ketamine, and nitrous oxide, and have also been produced by optogenetic inactivation of the thalamus (Lewis et al. 2015). Alpha oscillations are thought to be induced by GABAergic mediation of thalamocortical dynamics, as evidenced by computational models (Ching et al. 2010; Vijayan et al. 2013), animal studies (Baker et al. 2014; Flores et al. 2017), and human studies (Purdon et al. 2013; Akeju et al. 2014; Purdon, Sampson et al. 2015). In particular, alpha oscillation coherence appears to be dependent on rhythmic thalamic neuronal activity leading to abnormally synchronous alpha oscillations within thalamocortical loops (Ching et al. 2010). Therefore, alpha oscillations appear to reflect GABAergic inhibitory circuit function, and the coherence of these frontal alpha oscillations may reflect thalamocortical function.

While propofol and sevoflurane are the focus of this thesis work, studies have shown that anesthetic drugs induce highly structured brain oscillations that are drug- and mechanism-specific and are readily visible within the EEG (Purdon, Sampson et al. 2015). As a result, emerging research characterizing these anesthetic-specific effects on the brain has led to a new paradigm for monitoring brain states under general anesthesia and sedation using the EEG signatures (Purdon, Sampson et al. 2015).

**Monitoring Brain States Using the Electroencephalogram**

Under this new paradigm, one approach for minimizing anesthetic exposure in children would be to adjust anesthetic dosing using EEG-based brain monitoring (Akeju et al. 2015). Studies in adults have shown that general anesthetics induce structured EEG oscillations that reflect activity in specific neural circuits (Ching et al. 2010; Brown et al. 2011; Purdon et al. 2013; Purdon, Sampson et al. 2015). More specifically, the brain states induced by general anesthesia have been well-studied in adults through detailed characterizations of behavior and consciousness at varying drug levels in human volunteers (Purdon et al. 2013). In children, this powerful approach is not possible because children cannot ethically participate in volunteer
studies of dose-dependent responses to anesthetics due to safety concerns. As a result, studies to date have been in the setting of surgeries or procedures that require general anesthesia.

Nevertheless, it is important to study the age-specific effects of anesthetic drugs on the brain, because the findings in adults do not reliably translate to children. Commonly used depth-of-anesthesia monitors use various features of the EEG, such as power in certain frequency bands or degree of burst suppression, to compute an index between 0 and 100 to indicate level of consciousness and assess for adequate anesthesia (Davidson 2007; Palanca et al. 2009). These monitors typically interpret power at higher frequencies to indicate lighter levels of anesthesia or increased levels of awareness. However, these monitors, developed in adults with the goals of reducing intraoperative awareness and preventing anesthetic overdose (Palanca et al. 2009), have not been reliable in children (Davidson 2007). Moreover, despite extensive studies investigating general anesthesia-induced EEG patterns in adults, there have been far fewer studies in children.

Early studies of the pediatric EEG during general anesthesia largely focused on analyzing processed EEG parameters, including the bispectral index (BIS) (Bannister et al. 2001; Davidson et al. 2001; Davidson et al. 2005; Kim et al. 2005; Wodey et al. 2005; Tirel et al. 2006; Lo et al. 2009; McKeever et al. 2014), EEG power (Davidson et al. 2008; Lo et al. 2009), spectral edge frequency 90% (SEF90) (Davidson et al. 2008; Hayashi et al. 2012; McKeever et al. 2012; McKeever et al. 2014), and amplitude-integrated EEG (aEEG) (Davidson et al. 2008; McKeever et al. 2012; McKeever et al. 2014). Although the age groups studied varied widely, one common theme was that infants younger than 6 months of age had significantly different responses to general anesthesia: lower BIS values in infants (Davidson et al. 2001; Lo et al. 2009) and relative lack of responsiveness to changes in anesthetic concentration (Davidson et al. 2005; Hayashi et al. 2012). In addition, children greater than 6 months of age exhibited age-specific variation in the EEG parameters and BIS values (Davidson et al. 2005; Davidson et al. 2008; Hayashi et al. 2012). However, these measures were limited in that they did not investigate the structure of the anesthesia-induced oscillations. Total EEG power, SEF90, or aEEG are parameters that do not assess the frequency breakdown of an EEG signal; as a result, they may provide a biased representation of the changes in high amplitude oscillations at a given frequency, while concealing changes in smaller amplitude oscillations at other frequencies. There have been few studies exploring changes in power spectra over age with general anesthesia in children. In one study of sevoflurane-induced EEG power spectra in infants, subjects greater than 3 months of age were found to have a distinct increase in power in the 5-20 Hz frequency compared to those less than 3 months of age (Sury et al. 2014).
More recent studies in children have begun to characterize the general anesthesia-induced EEG patterns in children within the context of development, based on the paradigm of drug-specific EEG oscillations and EEG-based brain monitoring in adults (Akeju et al. 2015; Cornelissen et al. 2015). Specifically, sevoflurane-induced EEG oscillations have been found to vary with age in children and likely reflect neurodevelopmental processes (Akeju et al. 2015; Cornelissen et al. 2015). Briefly, Akeju et al. (2015) showed that frontal EEG power varied as a function of age in patients 0 to 28 years of age, increasing from infancy to approximately 6 years of age before declining through the adolescent years, and that frontal alpha coherence was not observed in patients less than 1 year of age. Cornelissen et al. (2015) investigated EEG dynamics in infants 0 to 6 months of age, and demonstrated that alpha oscillations were absent in infants 0-3 months of age, emerged around 3-4 months of age, and remained widespread at 6 months, in contrast to adults. Together, these studies established that the EEG oscillations during general anesthesia in children vary significantly with age, likely due to neurophysiological differences between infants, children, and adults.

**Study Objectives**

Given that the nervous system undergoes significant changes from birth to adulthood (Tau & Peterson 2010), it is not surprising that anesthesia-induced EEG oscillations in children differ significantly from those in adults (Davidson 2007; Tirel et al. 2008; Davidson et al. 2008; Lo et al. 2009; McKeever et al. 2012; Hayashi et al. 2012; Sury et al. 2014; Cornelissen et al. 2015; Akeju et al. 2015). The purpose of this thesis work was to characterize the early changes in general anesthesia-induced EEG oscillations in children and explore how these changes may reflect neurodevelopmental changes, particularly in GABAergic circuitry and the thalamocortical connections implicated in propofol- and sevoflurane-induced unconsciousness. This thesis work will be presented as two separate parts. In Part 1, the EEG dynamics during propofol general anesthesia in children were hypothesized to vary with age in a manner similar to sevoflurane. A prospective observational study was performed to characterize and compare age-dependent propofol EEG dynamics in children 0-21 years of age. Given the distinct changes observed in the propofol- and sevoflurane-induced EEG in children less than 1 year of age, Part 2 of this thesis aimed to better characterize the age-related EEG changes in young children. In Part 2, the EEG dynamics during sevoflurane general anesthesia in children 0-3 years of age were hypothesized to change with age in a way that could lend insight into early neurodevelopmental changes. A prospective observational study was performed to describe these age-related changes in spectral power, coherence, and spatial distribution of slow and alpha oscillations.
By understanding how the effects of general anesthesia change during brain development, we may be able to use EEG monitoring to develop more effective ways of tracking and establishing appropriate brain states in pediatric patients, and in doing so, enhance anesthetic safety. Furthermore, these anesthetic-specific signatures in the EEG may provide a unique opportunity to noninvasively track human brain development, with a focus on specific neural circuits, such as the GABAergic circuits induced by propofol or inhaled anesthetics.
PART 1: PROPOFOL-INDUCED EEG OSCILLATIONS IN CHILDREN 0-21 YEARS OF AGE

METHODS

Patient Selection

This prospective observational study was approved by the Human Research Committee at Massachusetts General Hospital. We collected a total of 155 cases from individuals between 0 and 21 years of age. Of these, we identified 150 cases in which propofol was administered as the sole primary anesthetic. We excluded patients who had neurological or psychiatric abnormalities, including autism, attention deficit hyperactivity disorder, seizures, and other congenital or psychiatric conditions (n=32). We also excluded cases for EEG artifacts and burst suppression (n=11), cases too short to identify a stable epoch without other drugs administered (n=4), and subjects who received the confounding adjunct drugs midazolam or scopolamine (n=6). We ultimately identified a total of 97 cases that contained a two-minute epoch of stable propofol infusion with no other anesthetic drugs given for at least five minutes prior to the epoch. Figure 1 summarizes patient selection, with inclusion and exclusion criteria. We analyzed patient characteristics for each age group, including age, gestational age, sex, weight, procedure type, and length of procedure. We also tested whether gestational age, sex, length of anesthesia, and propofol infusion rates were significantly different among age groups, using a Kruskal–Wallis One way Analysis of Variance (ANOVA) test on rank.

Data Collection

We recorded four-channel frontal EEG data using the SEDLine brain function monitor (Masimo Corporation, Irvine, CA). We selected time windows for analysis from the recorded EEGs using information from the electronic anesthesia record (Metavision, Dedham, MA). The concentrations of drugs administered to patients were manually recorded in the electronic anesthesia record by the anesthesia providers. For each patient, we identified a two-minute epoch with a stable propofol infusion rate. For patients induced with inhaled anesthesia (sevoflurane and/or nitrous oxide), this two-minute period occurred at least 5 minutes after cessation of the inhaled anesthetic. Two of the authors (J.M.L., K.T.) visually inspected all EEG data for each patient and manually identified epochs that were free of noise, artifacts, or segments of burst suppression for analysis.

Spectral Analysis

The power spectrum deconstructs a given EEG signal to provide the frequency distribution of power within a given range; the spectrogram represents the power spectra across
time (Figure 2). For each patient, we computed the power spectrum and visualized the spectrogram using the multitaper spectral analysis methods implemented in the Chronux toolbox in MATLAB (Mathworks, Natick, MA) (Percival & Walden 1993). The parameters used for the multitaper spectral analysis were: sampling frequency $F_s = 250$ Hz, window length $T = 2s$ with no overlap, time-bandwidth product $TW = 3$, and number of tapers $K = 5$. To calculate estimates of power spectra, we used an EEG derivation equally weighting the signals from the channels Fp1, Fp2, F7 and F8. Median power was calculated from the EEG spectrum of each patient within the following frequency ranges: slow (0.1-1 Hz), delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-31 Hz), and gamma (31-40 Hz). We modeled the total power and power in the slow and alpha oscillation bands as polynomial functions of age, using forward stepwise multiple linear regression analysis to select the polynomial order.

Fentanyl can induce EEG slow oscillations at high doses (Sebel et al. 1981). We therefore sought to analyze potential confounds related to fentanyl administration. To quantify potential interactions between fentanyl and age, we calculated the correlation between age polynomial terms (i.e. age, age$^2$) and fentanyl dose ($\mu$g/kg). To quantify the potential influence of fentanyl administration on slow oscillation power, we performed a regression analysis featuring the age polynomial terms and fentanyl dose. We used the statistical software R to perform these analyses.

In addition, we estimated group-level spectra and spectrograms from the selected epochs by taking the median across all patients within each of the following age groups: 4 months to 1 year old ($n = 4$), >1-7 years old ($n = 16$), >7-14 years old ($n = 30$), and >14-21 years old ($n = 47$). We also computed an age-varying spectrogram using overlapping moving windows (0.5 years) spanning a +/- 2 year age range in patients ranging from 1 to 21 years.

**Coherence Analysis**

Coherence represents the degree of correlation between the two signals $x(t)$ and $y(t)$ at a given frequency $f$, where a coherence of 1 indicates the two signals are perfectly correlated and a coherence of 0 indicates the two signals are not correlated. The coherence $C_{xy}(f)$ function between two signals $x$ and $y$ is defined as

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

where $S_{xy}(f)$ is the cross-spectrum between the signals $x(t)$ and $y(t)$, $S_{xx}(f)$ is the power spectrum of the signal $x(t)$, and $S_{yy}(f)$ is the power spectrum of the signal $y(t)$. For each patient, we computed the coherence between two bipolar frontal channels, F7 – Fp1 (left) and F8 – Fp2
(right), using the multitaper methods implemented in the Chronux toolbox in MATLAB (Percival & Walden 1993). The parameters used for the multitaper coherence analysis were: sampling frequency \( F_s = 250 \text{ Hz} \), window length \( T = 2 \text{s} \) with no overlap, time-bandwidth product \( TW = 3 \), and number of tapers \( K = 5 \). Median coherence was calculated from the EEG of each patient, within the frequency ranges defined above. We modeled frontal coherence in the slow, theta, and alpha bands as polynomial functions of age, and used forward stepwise multiple linear regression analysis to select the polynomial order. We then estimated group-level coherence and coherograms for the selected epochs by taking the median across all patients within each of the age groups specified above. We also computed an age-varying coherogram using overlapping moving windows (0.5 years) spanning a +/- 2 year age range in patients ranging from 1 to 21 years.

**Statistical Analysis**

We used frequency-domain bootstrap methods to determine the confidence intervals for the spectral and coherence estimates, and for differences in power and coherence between groups. We calculated 95% confidence intervals for each spectral and coherence estimate, as well as for differences between power spectra or coherences, using a bootstrap procedure. Briefly, bootstrap samples \( n=5,000 \) for the median spectrum, median coherence, and differences in spectrum or coherence were drawn from each group. Bootstrap confidence intervals were calculated using the percentile method (Efron & Tibshirani 1993). To take into account the spectral resolution of the power spectra estimates, for frequencies \( f>2W \), power or coherence between two groups was considered to have a statistically significant difference only if the significance threshold (95% confidence interval did not contain zero) was met for consecutive frequencies throughout a frequency interval greater than or equal to the spectral resolution \( 2W \). For frequencies \( 0\leq f\leq2W \), differences in spectral estimates were considered significant only if the significance threshold was met throughout a consecutive frequency range from zero to max \( f,W \) \( \leq 2W \) (Bokil et al. 2007; Purdon, Sampson et al. 2015).

Briefly, bootstrap samples for each regression model were constructed by adding normally-distributed errors to the fitted regression curve. The variance of the normally-distributed bootstrap errors was set equal to the residual variance of the original regression analysis. The regression relationship was then re-estimated for each bootstrap sample to construct the 95% confidence interval for the regression curve. Confidence intervals for differences in the regression curves were estimated by taking the difference in regression curves from randomly drawn bootstrap samples from each group being compared. Power or
coherence between two groups was considered to have a statistically significant difference if the bootstrap 95% confidence interval of the difference did not include zero. All bootstrap analyses were computed using MATLAB.
RESULTS

Analysis of Patient Characteristics

Table 1 summarizes the characteristics of patients included in the study, as well as the propofol infusion rates and fentanyl doses administered prior to the chosen epoch. Gestational age, sex, length of anesthesia, and propofol infusion rates were not significantly different between age groups (Kruskal–Wallis One way ANOVA test on rank, p>0.05).

Power Spectra Analysis

For patients greater than 1 year old, the spectra and spectrograms show an EEG structure that is qualitatively similar regardless of age, featuring slow-delta and alpha oscillations (Figure 3A-H). Total EEG power (0.1-40 Hz) peaked at approximately 8 years old and subsequently declined with increasing age (Figure 3I). Multiple linear regression analysis showed a significant model fit for total EEG power (Figure 3I).

We compared the median spectra of the following age groups: 4 months to 1 year old, >1-7 years old, >7-14 years old, and >14-21 years old (Figure 4). We found that a distinct increase in power in the alpha oscillation frequency range was not apparent until approximately 1 year of age (Figure 4). Instead, there appeared to be an increase in spectral power over a broader frequency range (Figure 4A, E). For patients greater than 1 year old, the spectra and spectrograms showed EEG structures that were qualitatively similar with distinct peaks in the slow and alpha oscillation frequency ranges (Figure 4B-D, 4F-H). Statistically significant differences in power between age groups are reported in Table 2.

Coherence Analysis

We found age-related variation in the EEG coherence and coherograms during propofol-induced unconsciousness (Figure 5). In patients 1-21 years old, we observed coherent frontal alpha oscillations (Figure 5B-D, 5F-H), which were not seen in patients less than 1 year old (Figure 5A, E). We also observed that slow coherence increased with age, particularly in the adolescent years (Figure 5D, 5H). For patients greater than 1 year old, the coherence and coherograms showed EEG structures that were qualitatively similar, with prominent peaks in the alpha oscillation frequency range (Figure 5B-D, 5F-H). Statistically significant differences in power between age groups are reported in Table 2.
**Slow and Alpha Oscillations**

To further explore the age-related variations in frontal power and coherence, we investigated age-related changes in the slow and alpha oscillations, which are prominent during propofol-induced unconsciousness. We compared multiple regression models characterizing slow and alpha oscillation power across age (Figure 6). Frontal slow oscillation power peaked at approximately 11.6 years of age (95% confidence interval, 10.7-12.5 years; Figure 6), whereas frontal alpha oscillation power peaked at approximately 7.3 years of age (95% confidence interval, 6.5-8.2 years; Figure 6). The difference between these peak ages was statistically significant (95% confidence interval, 3.0-5.5 years). Alpha oscillation power was greater than slow oscillation power from 3.6 to 5.3 years of age, and slow oscillation power was greater than alpha oscillation power from 10.5 to 20.3 years of age (95% confidence interval, bootstrap analysis).

We found no evidence of age dependence in fentanyl administration: the correlation coefficient between age and fentanyl dose was -0.029, and the correlation coefficient between age$^2$ and fentanyl dose was 0.011. These correlation coefficients were not statistically significant. When fentanyl dose was added as a regressor to the model for slow oscillation power, we found that fentanyl dose did not have a significant association with slow oscillation power (coefficient = -1.08, 95% confidence interval, -2.28-0.11, p=0.08; equivalent to ~1 dB of power). This suggests that fentanyl dose did not have a significant effect on slow oscillation power in this study.

We also compared multiple regression models characterizing slow and alpha coherence across age (Figure 7). Slow coherence appeared to increase linearly between 1 and 21 years of age (Figure 7A), whereas alpha coherence peaked at 8.9 years of age (95% confidence interval, 7.4-12.2 years; Figure 7B). Alpha coherence was significantly greater than slow coherence for ages 2.6 to 14 years (95% confidence interval, bootstrap analysis; Figure 7C).

**Infants Under 2 Years of Age**

Because we observed qualitatively significant changes between the 4 month to 1 year and 1 year to 7 year age groups, we decided to examine this transition in more detail by comparing patients between 4 months and 1 year old and patients between 1 year and 2 years old (Figure 8). For patients less than 2 years of age, we consistently observed slow (0.1-1 Hz) oscillations in all subjects (Figure 8A, 3A, 3E). However, the power spectrum in subjects less than 1 year old illustrates the relative absence of well-defined alpha (8-13 Hz) oscillations, instead showing oscillations over a broader and faster frequency range, spanning approximately
12 to 25 Hz. Quantitatively, EEG power is significantly greater in the 1 to 2 year age group relative to the 4 month to 1 year age group for the following frequency ranges: 0 – 15.14 Hz and 20.51 – 33.69 Hz (95% confidence interval, bootstrap analysis; Figure 8A).

We also found that although frontal alpha power seemed to appear at about 5 months of age (Appendix A1), frontal alpha coherence was not apparent until between 1 year and 2 years of age (Figure 8B, Appendix A2). Frontal coherence was significantly greater in the 1 to 2 year age group relative to the 4 month to 1 year age group over a frequency range of 6.35 – 11.72 Hz (95% confidence interval, bootstrap analysis; Figure 8B).

**Age-Varying Frontal Spectrogram and Coherogram**

In order to better represent the age-related changes in the propofol-induced EEG over childhood development, an age-varying frontal spectrogram and coherogram were computed for ages 1-21, as shown in Figure 9. The frontal EEG structure is qualitatively similar across age after 1 year of age, compromised of prominent slow oscillations and frontally coherent alpha oscillations (Figure 9). At the same time, EEG power varies as a function of age and development, with beta-gamma power visibly declining with age, and alpha oscillation power decreasing by approximately 16 years of age (Figure 9).
**METHODS**

*Patient Selection*

This prospective observational study was approved by the Boston Children’s Hospital Institutional Review Board (Protocol Number P000003544), and the study was classified as a “no more than minimal risk” study. Informed written consent was obtained from the parents or legal guardians of the patient prior to each study. Children scheduled for an elective surgical procedure at Boston Children’s Hospital from December 2011 to August 2016 were recruited for inclusion in this study. I assisted with the recruitment of patients from July 2015 through June 2016; the remaining patients in this group were recruited by Dr. Cornelissen. Children who were eligible for the study were between 0 and 3 years of age and required surgery below the neck. Children were clinically stable on the day of surgery and categorized as American Society of Anesthesiologists’ physical status I or II. Exclusion criteria included genetic or metabolic disorders thought to influence brain development, diagnosis of neurological disorders, developmental delay, diagnosis of a cardiovascular disorder, and birth at <32 weeks post-menstrual age. A total of 106 patients were recruited for this study. Data from 91 patients were analyzed for the purposes of this study; each subject was studied one time. Figure 10 summarizes patient selection, with inclusion and exclusion criteria.

*Anesthetic Management*

Each patient was induced with sevoflurane alone or a combination of sevoflurane and nitrous oxide. In some cases, a propofol bolus was given to increase anesthetic depth, either before surgical incision or airway placement (n=44). The airway was secured by endotracheal intubation (n=62) or laryngeal mask airway (n=24). In a few cases, a face mask was used for the case (n=5). Seven patients were given midazolam as a premedication on the day of surgery.

Anesthesia was maintained with sevoflurane +/- nitrous oxide with air and oxygen, at a level that was determined to be clinically appropriate by the anesthesiologist. During maintenance, the median end-tidal sevoflurane concentration for patients was 2.4% (Table 3, 95% CI: 1.3-3.5).

*Data Collection*

We recorded 33- or 41-channel EEG using an EEG cap (WaveGuard EEG cap, Advanced NeuroTechnology, Enschede, Netherlands). The electrodes were positioned
according to the modified international 10/20 electrode placement system. For 33-channel EEG, recordings from the following electrodes were obtained: Fz, FPz, FP1, FP2, F3, F4, F7, F8, FC1, FC2, FC5, FC6, Cz, CPz, C3, C4, CP1, CP2, CP5, CP6, Pz, P3, P4, P7, P8, T7, T8, M1, M2, POz, Oz, O1 and O2. For 41-channel EEG, recordings were obtained from the following additional recordings: AF7, AF8, PO7, PO8, FT7, FT8, TP7, and TP8. For both 33- and 41-channel EEG, the reference electrode was located at Fz and the ground electrode at AFz. The impedance was kept to a minimum by applying an EEG skin prep gel (Nuprep, D.O. Weaver and Company, Aurora, CO, USA) prior to EEG cap placement. A conductive EEG gel was used to optimize the conductivity of EEG recordings (OneStep Cleargel, H+H Medical Devices, Münster, Germany). An Xltek EEG recording system (EMU40EX, Natus Medical Inc., Ontario, Canada) was used to record EEG activity from 0 to 500 Hz. Signals were digitized at a sampling rate of 1024 Hz (n=86) or 256 Hz (n=5), with a resolution of 16-bit.

We collected demographic information and clinical information including age, gender, weight, past medical history, surgical procedure and anesthetic management from the electronic medical records and the in-house Anesthesia Information Management System (AIMS). End-tidal sevoflurane, nitrous oxide, and oxygen were obtained from the anesthetic monitoring device (Drager Apollo, Drager Medical Inc., Telford, PA) to a recording computer in real-time using the ixTrend Express software (ixellence, Wildau, Germany) at 1 data point per second. Body movement and handling of the EEG cap were recorded using a video camera that was time-locked to the EEG recording (Xltek DSP270x, Natus Medical Inc., Ontario, Canada).

**EEG Analysis**

Data was pre-processed before analysis using Natus Neuroworks (Natus Medical Inc., Ontario, Canada) and MATLAB (Mathworks, Natick, MA). The electrodes M1 and M2 were excluded from final analysis due to poor surface-to-skin contact for the majority of subjects. For improved spatial resolution, nearest-neighbor Laplacian transformation of each EEG electrode was derived by differentially weighting the contribution of neighboring electrodes using inter-electrode distances. An anti-aliasing filter (low-pass filter with a cut-off frequency of 80 Hz) was applied to the EEG data, and the data was down-sampled to 256 Hz.

For each subject, we selected 5-minute time epochs from the recorded EEGs that reflected maintenance of surgical state of anesthesia and occurred at least 10 minutes after procedure start or surgical incision. Specifically, time epochs were selected for which end-tidal sevoflurane concentration was stable and did not change by more than 0.1%. EEG electrode recordings identified to have noise or artifact by visual inspection were excluded from analysis.
Two authors (J.M.L., L.C.) visually inspected all EEG data for each patient and manually identified epochs that were free of noise and artifacts for analysis.

**Spectral Analysis**

For each patient, we computed the power spectrum and visualized the spectrogram using the multitaper spectral analysis methods implemented in the Chronux toolbox in MATLAB (Mathworks, Natick, MA) (Bokil et al. 2010). The parameters used for the multitaper spectral analysis were: sampling frequency Fs = 250 Hz, window length T = 2s with 1.9s overlap, time-bandwidth product TW = 2, and number of tapers K = 3.

Subjects were divided into groups by postnatal age as follows: (i) 0-3 months (n=17), (ii) 4-6 months (n=23), (iii) 7-9 months (n=8), (iv) 10-14 months (n=17), (v) 15-17 months (n=7), and (vi) 18-40 months of age (n=19). For each subject, median spectrograms and spectra were calculated, and group-level spectrograms and spectra were estimated by taking the median across all patients within each of the above age groups. In order to better visualize the spectral trends over age, an age-varying spectrogram was computed using overlapping moving windows (1 month) spanning a +/-3 month age range in patients ranging from 0 to 40 months of age. To better estimate the expected changes in the spectrogram with age, a Kalman filter and fixed interval smoothing algorithm were applied to the age-varying spectrogram.

In order to characterize the spatial distribution of the EEG oscillations, group-averaged spectrograms were calculated for each electrode within the following frequency bands: slow (0.1-1 Hz), delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-25 Hz), and gamma (25-40 Hz). Then, mean power spectra were averaged over each frequency band for each given age group. These values were represented using a 3D interpolation of the electrode montage using the topoplot function in EEGLab (Delorme & Makeig 2004).

**Coherence Analysis**

Frontal coherence analysis was performed as described in Part 1 by computing the coherence between two bipolar frontal channels F7 – Fp1 (left) and F8 – Fp2 (right) using multitaper methods with the following parameters: Fs = 250 Hz, T = 4s with 3s overlap, TW = 4, and K = 7. Individual coherence plots and coherograms were estimated, and group-level coherograms were estimated by taking the median across all subjects within each age group. In order to characterize the coherence trends over age, an age-varying coherogram was computed using overlapping moving windows (1 month) spanning a +/-3 month age range in patients.
ranging from 0 to 40 months of age. The Kalman filter and fixed interval smoothing algorithm were also applied to the age-varying coherogram.

Global coherence analysis was also performed using previously described methods (Cimenser et al. 2011; Purdon et al. 2013). Each time epoch was divided into non-overlapping 2-second windows, and the median was taken over 10 windows of the real and imaginary parts of each entry in the cross-spectral matrix (Cimenser et al. 2011; Purdon et al. 2013). An eigenvalue decomposition analysis of the cross-spectral matrix at each frequency $f$ was performed:

$$C(f) = U(f)\Lambda(f)U(f)^H,$$

where $U(f)^H$ is the complex conjugate transpose of $U(f)$ and a unitary matrix, where the $ith$ column corresponds to the eigenvectors at frequency $f$, and $\Lambda(f)$ is a diagonal matrix containing the corresponding eigenvalues, such that $\Lambda_{ii}(f) = S_i(f)$. Global coherence of the EEG signal is defined as the ratio of the largest eigenvalue to the sum of eigenvalues:

$$C_{global}(f) = \frac{|S^Y_1(f)|}{\sum_{i=1}^N S^Y_i(f)},$$

where $S^Y_i(f)$ represents the $ith$ eigenvalue and $N$ indicates the number of electrodes (Cimenser et al. 2011). A high global coherence value suggests synchronized neural activity at a given frequency $f$. The eigenvector $u_1(f)$ that corresponds to the largest eigenvalue $S^Y_1(f)$ is the principal mode of oscillation at a given frequency $f$, and the coherence value was determined by taking the absolute square of this eigenvector (Purdon et al. 2013).

For this study, global coherence was computed at each frequency for the given 5-minute time epochs, and median global coherence was computed for each age group by taking the median was taken across subjects within each group. Spatial coherence within each frequency band was calculated by taking the median across the frequency band for each electrode, and group-level spatial distribution of coherence was determined by taking the median across subjects. These values were represented using a 3D interpolation of the electrode montage using the topoplot function in EEGLab (Delorme & Makeig 2004).

**Statistical Analysis**

Statistical analyses were performed using R v3.4.3 with MedOr, moments, and tidyverse packages (R Core Team 2013) and MATLAB (Mathworks Inc., Natick, MA). The polyfit function in MATLAB was used to model the relationship between age and power or between age and coherence, and the polyconf function was used to compute 95% confidence intervals. Frequency-domain bootstrap methods ($n = 10,000$) were used, as described above, to estimate
the best-fit regression functions, differences between regression functions, and their 95% confidence intervals to determine statistical significance. Paired-subject bootstrap methods were used to compare spectral estimates between electrode locations within the same subject (e.g. frontal vs. occipital). All bootstrap analyses were computed using MATLAB.

*Use of Previously Published Data*

A subset of the subjects used in this analysis have been studied with results reported in previous studies, specifically investigating sevoflurane-induced EEG patterns in infants 0-6 months of age (n=36, Cornelissen et al. 2015) and EEG discontinuity or suppression during general anesthesia in children 0-3 years of age (n=68, Cornelissen et al. 2017). The MATLAB codes used in this study are available through the former of these studies, and the codes can be found online in *eLife* (Cornelissen et al. 2015).
RESULTS

Analysis of Patient Characteristics

Table 3 and 4 summarize the demographics and characteristics of patients included in the data analysis. Weight at study was positively correlated with age at study (Table 3, Pearson's correlation coefficient 0.9, p<0.001), and significantly differed among age groups (Table 4, Kruskal–Wallis One way ANOVA test on rank, p<0.05). Gestational age (or postmenstrual age at birth), sex, procedure type, duration of anesthesia, and end-tidal sevoflurane were not significantly different among age groups (Table 3, Kruskal–Wallis One way ANOVA test on rank, p>0.05).

Age-Related Changes in Spectral Power

Spectral power analysis of the frontal EEG in children during maintenance of sevoflurane-induced anesthesia showed distinct age-related changes (Figure 11). Review of individual spectrograms showed prominent slow (0.1-1 Hz) and delta (1-4 Hz) oscillations at all ages; theta (4-8 Hz) and alpha (8-12 Hz) oscillation power emerged with increasing age (Figure 11A). Based on these observed age-related changes, the subjects were divided into six age groups: 0-3 months (n=17), 4-6 months (n=23), 7-9 months (n=8), 10-14 months (n=17), 15-17 months (n=7), and 18-40 months (n=19).

In children 0-3 months of age, the sevoflurane-induced EEG structure predominantly featured slow-delta (0.1-4 Hz) oscillations (Figure 11A). Theta and alpha oscillations began to emerge around 4 months of age and appeared to persist with age (Figure 11A). In order to better represent these evolutions in the sevoflurane-induced EEG, an age-varying frontal spectrogram was computed as shown in Figure 11B. Again, slow-delta oscillations are prominent at all ages, while theta and alpha oscillations emerged around 4 months of age and became more prominent with age (Figure 11B). Group-level spectrograms and spectra can be found in Appendix B1. Because slow, delta, and alpha oscillations were the predominant features of the sevoflurane-induced EEG, polynomial fits were estimated for frontal slow, delta, and alpha oscillation power across age (Figure 11C-E). Frontal slow and delta oscillations, which were present from birth, showed a steady increase in power with increasing age (Figure 11C-D). In contrast, frontal alpha oscillations showed a marked increase in power at approximately 3 or 4 months of age, and subsequently increased more steadily with age (Figure 11E).
**Spatial Distribution of Sevoflurane-Induced EEG Oscillations by Age Group**

To investigate the spatial distribution of sevoflurane-induced EEG oscillations, topographic plots were computed within the slow, delta, theta, alpha, beta (12-25 Hz), and gamma (25-40 Hz) frequency bands for each age group (Figure 12). Slow, delta, and theta oscillations were globally distributed across the scalp for all age groups (Figure 12). In contrast, alpha oscillations, which emerged around 4 months of age with a global distribution, began to develop frontal predominance around 7-9 months of age (Figure 12). Power in the beta and gamma frequency bands was low across all age groups.

In order to quantify this shift in spatial distribution of EEG power with age, frontal and occipital power were calculated for slow, delta, and alpha frequency bands across age (Figure 13). As alpha oscillations were most predominant at frontal electrodes F3 and F4, frontal electrode F3 and occipital electrode O1 were used to assess this frontal-occipital power difference. Frontal alpha oscillation power was significantly greater than occipital alpha power from about 7 months and older (Figure 13C, F). This quantitative data supports the observation from visual data that alpha oscillations become anteriorized at approximately 7-9 months of age and persist in older age groups (Figure 12).

**Age-Related Changes in Coherence**

Analysis of frontal EEG coherence and coherograms in children during sevoflurane-induced unconsciousness showed age-related variation. Individual representative coherograms are shown in Figure 14A. At 2 and 3 months of age, the sevoflurane-induced EEG showed minimal slow-delta frontal coherence (Figure 14A). By 9 months of age, there was a marked absence of frontal coherence in slow-delta or alpha oscillations (Figure 14A). At 11 months, frontal coherence in the alpha frequency band began to emerge and became increasingly coherent with increasing age (Figure 14A). At 16 months and greater, alpha oscillations were highly coherent frontally (Figure 14A). Slow-delta oscillations remained frontally incoherent for 9 months and greater (Figure 14A). In order to represent these age-related evolutions in frontal EEG coherence, an age-varying frontal coherogram was computed as shown in Figure 14B. Frontal slow-delta coherence can be observed from birth to approximately 6 months, thereafter declining with age; frontal alpha coherence developed at about 10 months of age and continued to increase with age (Figure 14B). Group-level median coherograms and coherence plots can be found in Appendix B2.

Polynomial fits were estimated for slow, delta, and alpha frontal coherence across age (Figure 14C-E). Frontal coherence within the slow and delta oscillation frequency bands were
coherent at birth but steadily declined with age until about 6 months of age, reaching a plateau (Figure 14C-D). In contrast, frontal alpha oscillations were incoherent at birth, but emerged at about 10 months of age and continued to increase with age (Figure 14E). Of note, although alpha oscillations were prominent in the spectrogram beginning at 4 months of age, they did not begin to develop frontal coherence until about 10 months of age.

In addition to investigating frontal coherence, global coherence was analyzed to investigate coherence across multiple EEG electrodes. Topographic maps of coherence were computed for slow, delta, theta, and beta frequency bands within each age group (Figure 15). Global coherence analysis identified strong coordinated alpha oscillations in the frontal leads in subjects 10 months or greater, with a global coherence projection weight of approximately 0.3 (Figure 15D-F). In contrast, although slow-delta oscillations were frontally coherent in infants 0-6 months of age, global coherence was weak across slow, delta, theta, and beta oscillations across all age groups (Figure 15A-C). This EEG pattern of incoherent slow-delta oscillations and frontally coherent alpha oscillations during sevoflurane general anesthesia in children greater than 10 months of age is consistent with the EEG pattern observed in adults during propofol general anesthesia (Cimenser et al. 2011; Purdon et al. 2013).
DISCUSSION

Summary of Main Findings

In this study, we found age-related changes in the EEG power spectra and coherence during propofol-induced unconsciousness in children 0-21 years of age (Part 1) and during sevoflurane-induced unconsciousness in children 0-3 years of age (Part 2). In Part 1, the propofol-induced EEG changed significantly with age:

(1) Total frontal EEG power (0-40 Hz) increased over the first several years of life, peaking around 8 years of age, followed by a decline in the adolescent years (Figure 3);

(2) In infants (<1 year old), the EEG consisted mainly of slow-delta oscillations, and alpha-beta oscillations began to appear at approximately 5 months of age, but did not become coherent until approximately 1 year of age (Figure 8, Appendix A1-2);

(3) In children >1 year old, the structure of the propofol-induced EEG oscillations was qualitatively similar for patients from 1 year of age through adulthood, featuring slow-delta and frontally coherent alpha oscillations (Figure 9).

Given these striking changes in the propofol-induced EEG in infants less than 1 year of age, Part 2 of this thesis work focused on young children 0-3 years of age in order to gain a more detailed understanding of the anesthesia-induced EEG in young children and its association to early development of the nervous system. In Part 2, spectral power and coherence analysis of the sevoflurane-induced EEG and its spatial distribution showed that:

(1) Slow-delta oscillations were present at all ages (Figure 11);

(2) Theta and alpha oscillations emerged at approximately 4 months of age (Figure 11);

(3) Alpha oscillations increased in power from 4 to 10 months of age, then plateaued and persisted with age (Figure 11);

(4) Alpha oscillations anteriorized at approximately 7 months of age, and this frontalization of alpha power persisted with age (Figure 12-13);

(5) Alpha oscillations became frontally coherent at approximately 10 months of age, and this frontal coherence persisted with age (Figure 14-15).

These age-related changes in EEG spectral power, spatial distribution, and frontal EEG coherence during general anesthesia likely reflect the development of the underlying neural circuits engaged by propofol and sevoflurane general anesthesia. Furthermore, an understanding of these developmental changes can help establish and refine a structured approach to brain monitoring in children receiving general anesthesia.
Study of EEG Dynamics as a Window into Neurodevelopment

These age-related changes in the general anesthesia-induced EEG likely reflect underlying neurodevelopmental processes that occur over childhood and adolescence (Akeju et al. 2015; Cornelissen et al. 2015). The human brain undergoes structural and functional development that begins during gestation but continues in the postnatal period through infancy, childhood, and adulthood (Tau & Peterson 2010; Insel 2010; Smyser et al. 2011; Borre et al. 2014). The development of the nervous system occurs in overlapping phases that vary according to the regions of the brain, but is mediated by biological processes including neuronal proliferation, apoptosis, migration, myelination, synaptogenesis, and neural pruning (Tau & Peterson 2010; Insel 2010). Much of neuronal proliferation and migration occur prenatally, while neural circuit development and myelination continue into adolescence (Insel 2010). Early postnatal brain development is characterized by marked myelination and synaptogenesis, with synaptic density peaking around 2 years of age (Andersen 2003; Tau & Peterson 2010; Borre et al. 2014). Following this process, the brain undergoes neural pruning and synaptic elimination in order to strengthen the newly formed neural circuits and reduce the number of synapses (Andersen 2003; Borre et al. 2014). In addition, studies in humans and non-human primates suggest there is an increase in prefrontal excitatory synapses in the postnatal period that peaks around 5 years of age and subsequently declines over adolescence to reach adult levels by about 18 years of age (Glantz et al. 2007; Insel 2010). This pattern is consistent with the age related variation presented in Part 1 of this thesis: an increase in anesthesia-induced EEG power over the first several years of life, peaking at about 8 years of age, followed by a decline in the adolescent years.

These EEG changes also appear to be consistent with developmental EEG changes observed during wakefulness and sleep. Notable age-related features of the EEG during wakefulness include: predominant slow-delta oscillations present from infancy that generally decrease in power with age (Marshall et al. 2002; Miskovic et al. 2015), decrease in total power and beta oscillation power during adolescence (Miskovic et al. 2015), and development of distinct theta oscillations at about 4 months of age, thought to be possible precursors to adult alpha oscillations, with peak frequency increasing throughout childhood and adolescence (Hagne et al. 1973; Marshall et al. 2002; Eisermann et al. 2013; Miskovic et al. 2015). During non-rapid eye movement (NREM) sleep in infants, there is an increase in EEG slow-delta power over the first few years of life that then declines during the adolescent years (Gaudreau et al. 2001; Jenni et al. 2004; Segalowitz et al. 2010; Feinberg & Campbell 2010; Sankupellay et al. 2011). In addition, during NREM stage N2, infants develop a peak in the low beta frequency
range (11-15 Hz) at 2-3 months, corresponding to the development of sleep spindles (Sankupellay et al. 2011; Chu et al. 2014). Of note, sleep spindles are generated in the thalamic reticular nucleus, and may be involved in formation of the thalamocortical network (Jenni et al. 2003; Sankupellay et al. 2011). These age-related changes in the EEG may be related to a variety of factors, including decline in gray matter volume due to synaptic pruning, increase in white matter, and changes in neuronal density or neurotransmitter levels (Segalowitz et al. 2010; Sankupellay et al. 2011; Feinberg & Campbell 2010; Chu et al. 2014; Miskovic et al. 2015).

Alpha Oscillations as an Indicator of GABAergic Inhibitory Circuit Function and Development

More specifically, the age-related EEG changes under general anesthesia likely reflect GABAergic inhibitory circuit function, and developmental changes in alpha oscillations may provide insight into maturation of the GABAergic circuit. EEG studies in adults have shown that coherent frontal alpha waves are a hallmark of the propofol-induced unconscious state (Purdon et al. 2013). Computational modeling studies of propofol-induced oscillations have suggested that GABAergic enhancement within cortical and thalamocortical circuits can produce the frontally coherent alpha oscillations observed under general anesthesia. In one model, cortical circuits containing both excitatory pyramidal neurons and inhibitory interneurons, in the absence of thalamic connections, could generate propofol-induced alpha-beta oscillations that were not coherent (McCarthy et al. 2008). However, a revised model that also incorporated thalamic reticular interneurons and thalamocortical relay neurons was found to produce coherent alpha oscillations (Ching et al. 2010). This suggests that thalamocortical connections are required to produce coherent propofol-induced alpha oscillations (Ching et al. 2010). Moreover, recent invasive neurophysiological studies in rodents show that propofol-induced frontal alpha oscillations involve both the thalamus and cortex (Baker et al. 2014; Flores et al. 2017). These alpha oscillations were found to be coherent after loss of consciousness (Flores et al. 2017).

Propofol-induced alpha oscillations showed an age-dependent time course between 1 and 21 years, with frontal alpha power peaking at about 8 years of age (Figure 3). As GABAergic mediation of thalamocortical connections between the thalamus and medial prefrontal cortex are thought to underlie the induction of alpha oscillations during propofol and sevoflurane general anesthesia (Flores et al. 2017), this pattern suggests that development of the GABAergic inhibitory circuit involving the prefrontal cortex peaks at about 8 years of age, with inhibitory synapses declining thereafter. Similarly, human prefrontal cortical pyramidal neuron dendritic spine density also peaks at approximately 7 years of age (Petanjek et al. 2017).
2011). While dendritic spines typically receive excitatory input, they are thought to develop in synchrony with GABAergic inhibitory synapses in the prefrontal cortex (Anderson et al. 1995).

We observed striking changes in the structure of the EEG during propofol- and sevoflurane-induced unconsciousness over the first year of life. In infants 0-3 months of age, the propofol- or sevoflurane-induced EEG consisted mainly of slow-delta oscillations, with a marked absence of alpha oscillations. Consistent with previous studies of infants receiving general anesthesia (Akeju et al. 2015; Cornelissen et al. 2015), we saw that alpha oscillations began to appear at approximately 4 months of age with both propofol and sevoflurane general anesthesia. At these ages, these oscillations did not exhibit the prominent, narrow peak typically observed in the alpha frequency range in adults, but instead spanned a broader range that included the low beta frequency range. While the neurophysiological basis for this difference is uncertain, this suggests that the inhibitory circuits between the prefrontal cortex and thalamus are still immature at this age. Of note, these alpha oscillations emerged at approximately the same age as sleep spindles (2-3 months), which are generated by GABAergic thalamic reticular neurons and are synchronized by corticothalamic projections (Steriade 2003), lending further support for the thalamocortical basis for alpha oscillations.

We also observed that these alpha oscillations became anteriorized at about 7 months of age, but did not become coherent until 10 months of age. The emergence of frontal alpha coherence at ~10 months of age likely reflects underlying further development of connectivity within frontal thalamocortical circuits. This interpretation is consistent with imaging studies showing that frontal thalamocortical functional connectivity does not develop until 1 year of age (Alcauter et al. 2014). These patterns are consistent with and perhaps explain previous studies that suggested a significantly different EEG pattern in infants less than 6 months to 1 year old, whether assessing quantitative EEG parameters or EEG power spectra under general anesthesia (Bannister et al. 2001; Davidson et al. 2001; Davidson et al. 2005; Davidson et al. 2008; Lo et al. 2009; Hayashi et al. 2012; McKeever et al. 2014; Sury et al. 2014).

Maturation of GABAergic inhibitory circuits within the cerebral cortex and the thalamus likely plays a role in mediating this thalamocortical functional connectivity (Zhang & Jones 2004; Zhang et al. 2014), as could development of diffusely-projecting calbindin-positive thalamocortical matrix cells that are thought to mediate coherent thalamocortical spindle oscillations during sleep (Jones 2001; Bonjean et al. 2012). In addition, we hypothesize that the development of incoherent propofol-induced alpha oscillations at about 4 months could reflect the development of inhibitory GABAergic transmission, possibly influenced by age-related changes in the expression levels of cation-chloride cotransporters NKCC1 and KCC2 (Hyde et
al. 2011; Myers et al. 2012), or functional maturation of GABAergic synapses within the cerebral cortex or thalamocortical circuit (Gonzalez-Burgos et al. 2014). Another population of neurons which plays an important role in the formation and maturation of the thalamocortical circuit consists of subplate neurons. Subplate neurons are a transient population of cells in the developing cerebral cortex that are involved in the development of cortical connections, cortical inhibition, and developmental plasticity (Kanold & Luhmann 2010). These neurons receive inputs from the thalamus during gestation and play an important role in the development of corticocortical and thalamocortical connections during early development (Kanold & Luhmann 2010). The subplate zone, a transient compartment of the fetal cerebral cortex, generally regresses during the last few weeks of gestation and early postnatal life, but studies suggest remnant subplate neurons can persist in certain regions of the brain, like the prefrontal cortex, for up to 6 months or even 1 year (Delalle et al. 1997; Kostovic & Jovanov-Milosevic 2006; Kanold & Luhmann 2010; Myers et al. 2012; Kostovic et al. 2014). Postnatally, while the subplate zone disappears, many subplate neurons survive as interstitial neurons in the subcortical white matter through adulthood (Kostovic et al. 2011; Judas et al. 2013; Kostovic et al. 2014). Although this population of neurons has not been fully characterized in humans, the early remnant subplate neurons that persist postnatally in the prefrontal cortex may in part influence the development and maturation of thalamocortical and corticocortical circuits and, thus, the observed alpha oscillations and coherence during general anesthesia.

Finally, anatomically, brain structure develops rapidly over the first year of life, as seen in autopsy studies (Brody et al. 1987; Kinney et al. 1988) and imaging studies (Welker & Patton 2012). One development is myelination of the anterior limb of the internal capsule from approximately 2-8 months of age by MRI (Welker & Patton 2012), with mature myelin observed on pathology by approximately 12 months of age (Kinney et al. 1988). Central frontal white matter myelination appears to begin around 11 months of age and continue through 24 months of age by MRI (Welker & Patton 2012). These developmental changes in neuroanatomy may reflect myelination and maturation of the thalamocortical fibers between the thalamus and the anterior lobes, which are contained in the anterior limb. Altogether, the various structural and functional changes within the thalamocortical circuit appear to loosely parallel the evolution of the alpha oscillations over the first several months of development. Figure 16 shows a hypothetical model representing the underlying neurodevelopmental changes in thalamocortical connectivity that underlie the age-related changes observed in alpha oscillations. Figure 17 shows a summary of postnatal brain development, specifically synapse formation and white
matter myelination, alongside developmental milestones and structural EEG changes in slow and alpha oscillations during sevoflurane general anesthesia.

**Clinical Implications: Use of EEG for Brain Monitoring in Children During General Anesthesia**

In addition to elucidating the natural development of neural circuits engaged by anesthetics, the EEG can be used to monitor the brain during general anesthesia. The age-dependent changes in propofol- and sevoflurane-induced EEG we report here are consistent with previous studies (Akeju et al. 2015; Cornelissen et al. 2015). General anesthesia maintained with propofol or sevoflurane are both associated with large slow-delta and coherent frontal alpha oscillations (Akeju et al. 2014). Accordingly, we saw that propofol and sevoflurane both showed qualitatively similar age-dependent changes in these oscillations. Sevoflurane also induces a theta oscillation not seen under propofol (Akeju et al. 2014), whose power and coherence also vary with age. The differences in age-varying oscillatory structure in propofol- and sevoflurane-induced EEG could reflect differences in the circuit- and receptor-level effects of these drugs. While propofol and sevoflurane both act at GABA_A receptors, sevoflurane also acts at a number of other receptors including NMDA receptors, serotonin receptors, and two-pore potassium channels (Akeju et al. 2014). As some neural circuits may be influenced differently depending on the molecular receptors or channels being affected, further characterization of the age-related differences in the EEG under propofol, sevoflurane, and other anesthetic drugs could inform our understanding of development within different receptor-dependent circuits.

The possibility of using EEG monitoring to track brain states guide anesthetic management is especially important given recent discussions concerning possible anesthetic-related neurotoxicity in young children. In Part 1 of this study, children tended to have greater power than adults in the beta and gamma band oscillations (13-40 Hz), which are often associated with lighter levels of anesthesia, and with muscle activity indicative of emerging consciousness. As a result, if applied to children, these monitors could therefore tend to misinterpret the increased high frequency power to suggest that patients are not adequately anesthetized, which in turn could lead clinicians to administer higher doses of an anesthetic than needed to maintain unconsciousness during general anesthesia.

An alternative to using depth-of-anesthesia indices is to use the unprocessed EEG and spectrogram to monitor brain states during general anesthesia and sedation (Bennett et al. 2009; Purdon, Sampson et al. 2015). Although the total EEG power in children varied with age, we found that the structure of propofol-induced EEG oscillations was qualitatively similar for
patients from 1 year of age through adulthood, featuring slow-delta and frontally coherent alpha oscillations. Given the qualitative similarity in the structure of propofol-induced EEG oscillations in children and adults, our results suggest that the spectrogram-based approach could fully be applied to monitor brain states in children greater than 1 year of age. Using this approach, anesthetic dosing could be titrated to administer enough anesthetic to adequately anesthetize the patient, yet not administer more than needed to maintain unconsciousness. Children less than 1 year of age show significantly different general anesthesia-induced EEG signatures, as the neural circuits engaged by the anesthetics are still developing. For children 4 months old or greater, it is likely that the EEG pattern of slow and alpha oscillations, though not coherent, could still be used to guide anesthetic management (Cornelissen et al. 2017). Ultimately, further investigation will be required to establish principled monitoring approaches in these very young patients.

In addition, monitoring for certain EEG features during anesthesia may help further guide management. For example, in adults, high doses of anesthesia can lead to an EEG pattern called burst suppression, where the EEG alternates between bursts of electrical activity and periods of isoelectricity (Purdon, Sampson et al. 2015; Fritz et al. 2016). Intraoperative burst suppression has been associated with an increased risk for postoperative delirium, long-term mortality, and reduced functional independence in adults (Soehle et al. 2015; Fritz et al. 2016). Burst suppression has also been observed in children during anesthesia (Davidson et al. 2008; Hayashi et al. 2012). Discontinuity events, which are a similar but subtly different EEG feature characterized by widespread EEG suppression with a smoother transition to large amplitude electrical activity, have been observed in young infants less than 1 year of age during sevoflurane general anesthesia (Davidson et al. 2008; Cornelissen et al. 2017). Discontinuity events appear to be more common in younger infants (Davidson et al. 2008; Cornelissen et al. 2017), and appear to generally occur with high sevoflurane concentrations, e.g. end-tidal sevoflurane > 3% during induction (Cornelissen et al. 2017). In addition to being a possible marker of excessive depth of anesthesia, burst suppression or discontinuity may indicate pre-existing subclinical neural dysfunction that makes the brain more sensitive to anesthetic drugs (Fritz et al. 2016). Burst suppression and discontinuity events during general anesthesia have not been studied in children as a predictor of long-term outcome, but may be important features to monitor moving forward (Cornelissen et al. 2017).

Furthermore, although this thesis work focused on age-related changes in the EEG in children without neurologic or psychiatric disorders, this work may have possible implications for patients with neurodevelopmental disorders with abnormalities in GABAergic circuit function or
excitatory-inhibitory imbalance, such as schizophrenia or autism (Le Magueresse & Monyer 2013). For example, both of these disorders have abnormal GABAergic signaling that may alter the EEG structure under general anesthesia and affect anesthetic requirement for maintenance of unconsciousness. Walsh et al. (2017) showed that patients with autism spectrum disorder were significantly more likely to experience burst suppression during propofol general anesthesia than neurotypical patients, despite tending to receive lower propofol doses. These results suggest that patients with autism may require lower doses of GABAergic anesthetics than neurotypical patients to achieve similar levels of sedation or unconsciousness (Walsh et al. 2017). Similar differences may hold true for patients with schizophrenia. These results also support the utility of EEG-based monitoring of brain states to titrate anesthetic dosing based on the individual patient’s anesthetic requirements, which may vary not only with age but also with any pre-existing neurological dysfunction, whether subclinical or clinical.

Finally, the GABAergic circuits engaged by anesthetic drugs play an important role in the regulation of critical periods during early development (Hensch 2005). Critical or sensitive periods are limited time windows during brain development, during which experience strongly influences or permanently alters neural circuits and their functions (Hensch 2005; Takesian & Hensch 2013; Werker & Hensch 2015). Changes in the excitatory-inhibitory circuit balance, mediated by the maturation of GABAergic circuits, trigger the onset of critical periods (Takesian & Hensch 2013; Werker & Hensch 2015). GABAergic agents like benzodiazepines were found to accelerate the onset of plasticity (Hensch 2005). As such, anesthetic drugs like propofol or inhaled anesthetics that enhance GABA inhibition may have a similar effect and accelerate critical period onset at certain doses or levels of exposure, potentially disrupting normal development and adversely influencing neurodevelopmental outcomes. In addition, since different systems may develop plasticity at different times and rates during development, the age at exposure may influence what aspects of neurocognitive function are affected. It is possible that the age-related changes seen in the propofol- and sevoflurane-induced EEG reflect critical period plasticity, and further characterization of these EEG changes, which reflect activity in the underlying neural circuits, may help track brain development and plasticity. Future studies to explore critical period plasticity in relation to early anesthetic exposure may provide further insight into the neural circuits involved and perhaps guide age- and mechanism-specific drug selection for anesthesia or sedation.
Case Illustration: Brain States are Visible in the EEG for Children During Anesthesia

To provide one example of the potential for using EEG-based monitoring in children, a pediatric case of propofol anesthesia for endoscopy and colonoscopy is reported (patient included in Part 1 analysis). In this case, the patient was maintained on a propofol infusion after a sevoflurane/nitrous oxide induction, and the endoscopy was well-tolerated. However, shortly after the colonoscopy was initiated, the patient began moving in response to the procedural stimulus, and a propofol bolus was given and the infusion rate was increased. The patient subsequently stopped moving and the remainder of the procedure was uncomplicated.

The spectrogram for this case is shown in Figure 18 and illustrates these two brain states: (1) Pattern 1, light sedation, where the patient began moving in response to the procedural stimulus, and (2) Pattern 2, deep sedation or general anesthesia, after an additional propofol bolus, where the patient was able to tolerate the procedure without movement. At the start of Pattern 1, the EEG is characterized by slow-delta oscillations and high frequency beta-gamma oscillations, reflective of the EEG pattern associated with lighter states of anesthesia. In contrast, at the start of Pattern 2, the EEG is shows the characteristic slow and alpha oscillations associated with propofol general anesthesia. This case illustrates that the different brain states are readily apparent on the EEG and suggests that the spectrogram could be used to guide anesthetic management in children.

Study Limitations

Part 1. A limitation of Part 1 of this study is that there were relatively few patients under the age of 1 year that were included in this analysis (n=4). As such, it is possible that the magnitude of the difference we observed between 4 month to 1 year old children and 1 to 2 year old children may not be representative of the larger population. However, the absence of coherent alpha oscillations in infants and the appearance of coherent alpha oscillations after 1 year of age that we observed are consistent with previous studies of the EEG under sevoflurane in children (Akeju et al. 2015; Cornelissen et al. 2015). These observations are also consistent with the EEG changes we observed in Part 2. Another limitation of this observational study is that the anesthetic management of patients was not controlled or standardized. As such, it is possible that differences in the anesthetic management of these patients may have influenced the observed differences in the EEG. However, this seems unlikely due to the minimal variation in clinical procedures and propofol infusion rates across the patients studied and the magnitude of the EEG changes observed in our data.
In particular, most of our data came from patients receiving propofol for esophagogastrroduodenoscopy and/or colonoscopy, and underwent relatively similar levels of procedural stimulation. Thus, in comparison to a more general pediatric surgical population, the patients we studied experienced highly consistent rates and patterns of propofol administration, fewer adjunct medications, without use of neuromuscular blocking agents, all of which improve the quality and consistency of the EEG data analyzed. Moreover, it seems unlikely that small variations in clinical management, pharmacokinetics, and/or pharmacodynamics could account for the magnitude of the EEG changes observed in our data, which show differences in slow and alpha power spanning ~10dB across the age range studied, equivalent to a ~3-fold difference in the size of these oscillations. Similarly, such clinical or pharmacologic variations are unlikely to explain the absence of alpha oscillation coherence in infants, because this is a prominent feature of propofol-induced unconsciousness in adults. Nonetheless, future studies that carefully characterize age-dependent dose-response relationships in the EEG alongside structured assessments of level of consciousness are warranted. Overall, the large number of patients studied (n=97) within this cross-sectional analysis and the largely consistent trend in EEG power and coherence over age suggest that the age-related EEG changes we observed during propofol-induced unconsciousness reflect neurophysiologic changes that occur during development.

**Part 2.** Similar to Part 1, the anesthetic management of subjects in this observational study was not controlled or standardized, and as such, it is possible that systematic age-dependent variation in anesthetic management influenced the observed differences in the EEG. However, the time epochs chosen for analysis reflect maintenance of surgical anesthesia, where subjects were clinically anesthetized and unconscious. Furthermore, given our current understanding of the anesthesia-induced EEG in adults and children and the magnitude of the age-related changes in EEG power and coherence, the observed differences are more likely to reflect underlying neurodevelopmental changes over childhood. In Part 2, most of the patients studied were male due to the high proportion of urological surgery patients enrolled. Although EEG properties can be influenced by gender, there was no statistically significant difference in proportion of males among the age groups. Lastly, skull thickness changes over development, with skull conductivity decreasing with age during childhood, and could affect EEG power. However, this decrease in skull conductivity should decrease measured EEG power and uniformly affect all frequencies <100 Hz (Hamalainen et al. 1993). In contrast, EEG power generally increased with age, and the frequency breakdown of the EEG structure changed with age.
**Future Directions**

Future research could lead in a number of interesting directions. First, the EEG measures developed through our studies could be tested in clinical studies to determine whether the use of these measures lead to better patient outcomes than standard approaches that do not use the EEG. Given the concerns over possible anesthetic-related neurotoxicity, studies could also test whether EEG-guided anesthetic management actually decreases anesthetic dosing in children. In addition, studies could test whether features like intraoperative burst suppression affects postoperative outcomes, including postoperative cognitive dysfunction, emergence delirium, and long-term cognitive outcomes, and whether certain populations are more vulnerable and at higher risk for burst suppression.

Second, we could investigate the effects of other anesthetics on the EEG in children, both to help establish standards for brain monitoring and to study the development of the underlying neural circuits. Although this work focused on GABAergic circuits, NDMA receptors also play an important role in nervous system development, and ketamine could be studied as an important moderator of these circuits in young patients. More importantly, there are two anesthetic drugs that have received attention as non-GABAergic agents that have shown neuroprotective effects in preclinical studies: dexmedetomidine and xenon (Alam et al. 2017). Dexmedetomidine, an alpha-2 adrenoreceptor agonist, is an anesthetic adjunct and sedative used in operating rooms and intensive care units that may be a safer alternative to inhaled anesthetics (Pinyavat et al. 2016; Alam et al. 2017). Xenon is a NMDA antagonist, but unlike other NMDA antagonists like ketamine, appears to be neuroprotective, likely because of a different mechanism of action: not by blocking the ion pore but by competing for co-agonist glycine binding site (Alam et al. 2017). Both dexmedetomidine and xenon have demonstrated neuroprotective effects in preclinical studies and may reduce possible neurotoxic effects associated with GABAergic anesthetic agents (Alam et al. 2017). There is limited data on the use of these agents in the developing human brain, but clinical studies are underway for the use of dexmedetomidine combinations in children (NCT02353182, NCT03089905). These anesthetic adjuncts will hopefully provide ways to ameliorate possible neurotoxicity from GABAergic anesthetic agents like propofol or sevoflurane. Nonetheless, EEG monitoring of brain states with these drugs and drug combinations will still be important to ensure adequate but not excessive anesthesia. With a better understanding of the EEG patterns and underlying neurophysiological mechanisms for these drugs, future studies may help optimize safe and effective anesthetic administration in these cases.
Third, our investigation of EEG activity under general anesthesia could be enhanced by the addition of simultaneous functional near-infrared spectroscopy (fNIRS) to investigate age-related changes in neurovascular coupling during general anesthesia. fNIRS has been used to measure oxygenated and deoxygenated hemoglobin concentrations in the cerebral cortex, and monitoring these hemodynamic changes has been studied as a way of tracking depth of anesthesia (Curtin et al. 2014; Leon-Dominguez et al. 2014; Hernandez-Meza et al. 2015; Yeom et al. 2017; Hernandez-Meza et al. 2018). Combined EEG-fNIRS recordings during general anesthesia could improve our understanding about the brain’s hemodynamic changes during induction of anesthesia, maintenance, and emergence, how those changes relate to EEG activity, and how those changes vary with age. Lastly, we could investigate the characteristic EEG patterns during general anesthesia in patients with underlying neurodevelopmental disorders (e.g. autism, schizophrenia) or other patients at risk for adverse neurodevelopmental outcomes, as mentioned briefly above (Walsh et al. 2017).

Conclusions

In summary, the identified age-related changes in EEG power and coherence provide a strong argument for a more specific and principled approach to monitoring brain states in pediatric patients. Further investigations may help establish the precise correspondence between the structure of EEG oscillations and neurological development, as well as facilitate the development of specific pediatric recommendations for anesthetic management. We expect that such an approach will improve anesthetic monitoring and inform personalized anesthesia care for children (Purdon, Zhou et al. 2015). Ultimately, we believe that a deeper understanding of the developing neural circuits engaged by anesthetics will provide a strong foundation to improve anesthesia care and brain monitoring in the pediatric population.
SUMMARY

In the United States, approximately 6 million children are placed under general anesthesia for surgical procedures each year, but at present, there is no well-defined neurophysiological basis to define adequate anesthesia in children. In adults, the brain states induced by general anesthetic drugs have been studied through detailed characterizations of behavior and consciousness at varying drug levels in volunteers. Far less work has been done in children, who cannot ethically participate in volunteer studies of anesthesia due to safety concerns. Furthermore, there have been concerns raised about potential neurotoxicity with early anesthetic exposure in young infants. While some studies have shown adverse long-term neurodevelopmental outcomes after early anesthetic exposure, recent studies suggest that single, short anesthetic exposures may not affect long-term outcomes. However, it is yet unclear how multiple or prolonged exposures to anesthesia may affect brain development.

In this study, we found that the EEG signatures induced by the GABAergic anesthetics propofol and sevoflurane exhibit age-related variation, likely reflecting underlying neurodevelopmental processes, including synapse formation, myelination, and neural pruning. In Part 1, we studied the propofol-induced EEG in children 0-21 years of age. Children greater than 1 year old were found to have a qualitatively similar EEG structure during propofol general anesthesia to adults, namely slow-delta oscillations (0.1-4 Hz) and frontally coherent alpha oscillations (8-13 Hz). In Part 2, a subsequent study was done to characterize EEG oscillations in young children 0-3 years of age during sevoflurane general anesthesia. In children 0-3 years old receiving sevoflurane general anesthesia, slow-delta oscillations were present throughout all ages. In contrast, alpha oscillations emerged around 4 months old, anteriorized around 7 months old, and became frontally coherent around 10 months old.

These age-related changes in the general anesthesia-induced EEG likely reflect the development of the underlying neural circuits engaged by propofol and sevoflurane, including the thalamocortical circuit. Clinically, these findings suggest an age-specific strategy to monitoring brain states for pediatric patients receiving general anesthesia. The identified changes may also provide insight into the neurophysiological basis for the anesthetized state, and facilitate the development of a more principled approach to monitoring brain states in pediatric patients, allow more precise targeting of anesthetic drug dosing to desired brain states, and thus enhance patient safety.
REFERENCES


Table 1. Characteristics of Patients Included in Propofol Analysis (n=97).

<table>
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<tr>
<th></th>
<th>&lt;1 year (n=4)</th>
<th>&gt;1-7 years (n=16)</th>
<th>&gt;7-14 years (n=30)</th>
<th>&gt;14-21 years (n=47)</th>
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<tr>
<td><strong>Age (years)</strong></td>
<td>0.6 (0.3-0.9)</td>
<td>4.5 (1.4 - 6.9)</td>
<td>11 (7.3-13.9)</td>
<td>17.3 (14-20.7)</td>
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<td><strong>Gestational Age at Birth (weeks)</strong></td>
<td>39 (35-40) (n=4)</td>
<td>40 (36-40) (n=12)</td>
<td>40 (36-40) (n=12)</td>
<td>40 (36-40) (n=17)</td>
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<td><strong>Sex (male), n (%)</strong></td>
<td>2 (50)</td>
<td>11 (68.8)</td>
<td>17 (56.7)</td>
<td>26 (55.3)</td>
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<tr>
<td><strong>Weight (kg)</strong></td>
<td>5.5 (5-8)</td>
<td>15 (9-28)</td>
<td>37 (21-80)</td>
<td>63 (35-106)</td>
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<td><strong>Procedure type, n (%)</strong></td>
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<td></td>
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<tr>
<td>Endoscopy – EGD</td>
<td></td>
<td>11 (68.8)</td>
<td>23 (76.7)</td>
<td>22 (46.8)</td>
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<td>EGD + Colonoscopy</td>
<td>1 (25)</td>
<td>3 (18.8)</td>
<td>7 (23.3)</td>
<td>23 (48.9)</td>
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<tr>
<td>EGD + Sigmoidoscopy</td>
<td>2 (50)</td>
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<td></td>
<td></td>
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<tr>
<td>Colonoscopy</td>
<td></td>
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<tr>
<td>Sigmoidoscopy</td>
<td></td>
<td>1 (.06)</td>
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<tr>
<td>MRI brain and lumbar puncture</td>
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<td>Inguinal hernia repair</td>
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<td><strong>Duration of anesthesia (min), median (range)</strong></td>
<td>67 (17-131)</td>
<td>24 (17-64)</td>
<td>22 (10-138)</td>
<td>29 (9-161)</td>
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<td><strong>Propofol Infusion Rate (µg kg⁻¹ min⁻¹), median (range)</strong></td>
<td>250 (200-300)</td>
<td>250 (200-333)</td>
<td>250 (250-444)</td>
<td>250 (120-300)</td>
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<td><strong>Fentanyl (µg kg⁻¹), median (range)</strong></td>
<td>1.13 (1-2)</td>
<td>0.98 (0.59-1.33)</td>
<td>0.79 (0.61-2.27)</td>
<td>0.83 (0.35-3.03)</td>
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Table 1. Characteristics of Patients Included in Propofol Analysis. We report the characteristics of subjects included in the analysis for each age group. *Gestational age was included for subjects who had this information documented in their medical records. For the purposes of this paper, a “full-term” birth as documented in the medical records was equated with 40 weeks gestational age at birth. We also report the weight-adjusted propofol infusion rate (µg kg⁻¹ min⁻¹) and fentanyl dose (µg kg⁻¹). Gestational age, sex, duration of anesthesia, and propofol infusion rates were not significantly different among age groups (Kruskal–Wallis One way ANOVA test on rank, p>0.05).
Table 2. Results of Statistical Analysis: Comparison Between Age Groups.

<table>
<thead>
<tr>
<th>Age Group Comparison</th>
<th>Power Spectra</th>
<th>Coherence</th>
</tr>
</thead>
<tbody>
<tr>
<td>4mo-1yr vs. 1-7yr</td>
<td>1-7yr &gt; 4mo-1yr, 0 – 39.55 Hz</td>
<td>1-7yr &gt; 4mo-1yr, 6.35 – 13.18 Hz</td>
</tr>
<tr>
<td>1-7yr vs. 7-14yr</td>
<td>7-14yr &gt; 1-7yr, 0 - 7.81 Hz; 1-7yr &gt; 7-14yr, 13.18 - 39.55 Hz</td>
<td>7-14yr &gt; 1-7yr, 0 – 8.30 Hz, 11.23 – 22.95 Hz, 32.23 – 38.09 Hz</td>
</tr>
<tr>
<td>7-14yr vs. 14-21yr</td>
<td>7-14yr &gt; 14-21yr, 0 - 39.55 Hz</td>
<td>14-21yr &gt; 7-14yr, 0 – 2.93 Hz</td>
</tr>
</tbody>
</table>

Table 2. Results of Statistical Analysis: Comparison Between Age Groups. Bootstrap analysis (95% confidence interval) was used to compare the power spectra and coherence between age groups. This table reports the frequencies for which there was a statistically significant difference in power or coherence between age groups, as well as which age group had greater power or coherence.
Table 3. Overview of Patients Included in Sevoflurane Analysis (n=91).

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n=91)</th>
<th>r</th>
<th>r²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation at birth (weeks), median (IQR)</td>
<td>39 (38 - 39)</td>
<td>0.03</td>
<td>0.001</td>
<td>0.8</td>
</tr>
<tr>
<td>Age at study (months), median (IQR)</td>
<td>9.6 (5.4 – 17.1)</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Weight at study‡ (kg), median (IQR)</td>
<td>9.4 (7.1 – 11.5)</td>
<td>0.9</td>
<td>0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (male), % (n)</td>
<td>81.3 (74)</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Duration of anesthesia (min), median (IQR)</td>
<td>103 (81.2 - 171)</td>
<td>-0.03</td>
<td>0.001</td>
<td>0.8</td>
</tr>
<tr>
<td>End-tidal sevoflurane (%), mean (SD)</td>
<td>2.4 (0.6)</td>
<td>-0.03</td>
<td>0.001</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 3. Overview of Patients Included in Sevoflurane Analysis (n=91). Mean or median are reported as appropriate. The correlation between age at study and the following characteristics are reported in this table: gestation at birth, weight at study, length of anesthesia, and end-tidal sevoflurane. ‡Weight at study was positively correlated with age at study. The remaining characteristics did not change significantly as a function of age. IQR, interquartile range; n, number; r, Pearson’s correlation coefficient; r², coefficient of determination; SD, standard deviation. (See Table 4 for patient characteristics stratified by age group.)
### Table 4. Characteristics of Patients Included in Sevoflurane Analysis (n=91).

<table>
<thead>
<tr>
<th>Demographics</th>
<th>0-3 mo (n=17)</th>
<th>4-6 mo (n=23)</th>
<th>7-9 mo (n=8)</th>
<th>10-14 mo (n=17)</th>
<th>15-17 mo (n=7)</th>
<th>≥18 mo (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months), median (IQR)</td>
<td>2.9 (2.6-3.5)</td>
<td>6.0 (5.4-6.1)</td>
<td>8.4 (7.9-9.6)</td>
<td>12.8 (11.3-14.0)</td>
<td>17.5 (16.6-17.6)</td>
<td>28.3 (20.3-33.9)</td>
</tr>
<tr>
<td>Gestational Age at Birth (weeks), median (IQR)</td>
<td>38.0 (37-39)</td>
<td>39.9 (38.3-39)</td>
<td>39.0 (38.6-39.4)</td>
<td>39.0 (39.0-39.0)</td>
<td>39.0 (38.9-39.0)</td>
<td>39.0 (34.0-39.0)</td>
</tr>
<tr>
<td>Sex (male), % (n)</td>
<td>76.5 (13)</td>
<td>91.3 (21)</td>
<td>50 (4)</td>
<td>88.2 (15)</td>
<td>85.7 (6)</td>
<td>79.0 (15)</td>
</tr>
<tr>
<td>Weight‡ (kg), median (IQR)</td>
<td>5.7 (4.8-6.1)</td>
<td>7.7 (6.8-8.2)</td>
<td>8.1 (8.0-9.0)</td>
<td>10.3 (9.2-11.4)</td>
<td>11.1 (10.8-12.0)</td>
<td>13.9 (12.4-15.2)</td>
</tr>
<tr>
<td>Procedure type, % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urological surgery</td>
<td>82.4 (14)</td>
<td>95.7 (22)</td>
<td>62.5 (5)</td>
<td>82.4 (14)</td>
<td>85.7 (6)</td>
<td>78.9 (15)</td>
</tr>
<tr>
<td>General surgery</td>
<td>17.6 (3)</td>
<td>4.3 (1)</td>
<td>37.5 (3)</td>
<td>17.6 (3)</td>
<td>14.3 (1)</td>
<td>21.1 (4)</td>
</tr>
<tr>
<td>Anesthetic Management</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of anesthesia (min), median (IQR)</td>
<td>103 (86-140)</td>
<td>94 (77-170)</td>
<td>148.5 (101-184)</td>
<td>95 (86-132)</td>
<td>133 (107-175)</td>
<td>112 (67-170)</td>
</tr>
<tr>
<td>Propofol, % (n)</td>
<td>64.7 (11)</td>
<td>47.8 (11)</td>
<td>62.5 (5)</td>
<td>29.4 (5)</td>
<td>100 (7)</td>
<td>42.1 (8)</td>
</tr>
<tr>
<td>Local anesthetics, % (n)</td>
<td>82.3 (14)</td>
<td>95.7 (22)</td>
<td>87.5 (7)</td>
<td>82.4 (14)</td>
<td>57.1 (4)</td>
<td>57.9 (11)</td>
</tr>
<tr>
<td>Opioids, % (n)</td>
<td>82.3 (14)</td>
<td>65.2 (15)</td>
<td>87.5 (7)</td>
<td>94.1 (16)</td>
<td>85.7 (6)</td>
<td>84.2 (16)</td>
</tr>
<tr>
<td>Neuromuscular blocking agents, % (n)</td>
<td>70.6 (12)</td>
<td>34.8 (8)</td>
<td>37.5 (3)</td>
<td>52.9 (9)</td>
<td>42.9 (3)</td>
<td>31.6 (6)</td>
</tr>
<tr>
<td>Maintenance epoch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-tidal sevoflurane (%), mean (SD)</td>
<td>2.3 (0.7)</td>
<td>2.6 (0.6)</td>
<td>2.3 (0.3)</td>
<td>2.5 (0.6)</td>
<td>2.7 (0.6)</td>
<td>2.3 (0.4)</td>
</tr>
</tbody>
</table>

Table 4. Characteristics of Patients Included in Sevoflurane Analysis (n=91). We report the characteristics of subjects included in the analysis for each age group. We also report the end-tidal sevoflurane (%) during anesthesia maintenance epoch chosen for
Weight was significantly different among age groups (Kruskal–Wallis One way ANOVA test on rank, p>0.05). Gestational age, sex, length of anesthesia, and end-tidal sevoflurane were not significantly different among age groups. IQR, interquartile range; SD, standard deviation.
Figure 1. Patient Selection for Propofol Analysis.

We collected 155 cases from individuals between 0 and 21 years of age. Of these, we identified 150 cases in which propofol was administered as the sole primary anesthetic during maintenance of anesthesia. We excluded patients who had confounding medical conditions, including autism, attention deficit hyperactivity disorder, history of seizures, and other congenital or psychiatric conditions (n=32). Finally, we also reviewed cases for electroencephalogram artifacts, burst suppression, or other confounding adjunct drugs administered, making it difficult to identify a clean two-minute segment of time during maintenance (n=21). We ultimately identified a total of 97 cases that contained a two-minute epoch of stable propofol infusion with no other anesthetic drugs given for at least five minutes preceding the epoch. ADD, attention deficit disorder; ADHD, attention deficit hyperactivity disorder. Adapted, with permission, from Lee et al. (2017), A Prospective Study of Age-Dependent Effects of Propofol on Frontal Electroencephalogram Dynamics in Children. Anesthesiology. 2017 Aug;127(2):293-306, Figure 1.
Figure 2. Construction of the Spectrogram. (A) A 10-s electroencephalogram (EEG) trace recorded under propofol-induced unconsciousness. (B) The electroencephalogram trace in A filtered into its two principal oscillations: the blue curve, an alpha (8 to 12 Hz) oscillation, and the green curve, a slow (0.1 to 1 Hz) oscillation. (C) The spectrum provides a decomposition of the electroencephalogram in A into power by frequency for all of the frequencies in a specified range. The range here is 0.1 to 30 Hz. Power at a given frequency is defined in decibels as the
10 times the log base 10 of the squared amplitude. The *green horizontal line* underscores the slow-delta frequency band and the *blue horizontal line* underscores the alpha frequency band used to compute the filtered signals in $B$. The median frequency, 3.4 Hz (*dashed vertical line*), is the frequency that divides the power in the spectrum in half. The spectral edge frequency, 15.9 Hz (*solid vertical line*), is the frequency such that 95% of the power in the spectrum lies below this value. (D) The three-dimensional (3D) spectrogram (compressed spectral array) displays the successive spectra computed on a 32-min electroencephalogram recording from a patient anesthetized with propofol. Reprinted, with permission, from Purdon et al. (2015), Clinical Electroencephalography for Anesthesiologists: Part I: Background and Basic Signatures, Anesthesiology. 2015 Oct;123(4):937-960, Figure 3.
Figure 3. Age-related variation in spectra, spectrograms, and total electroencephalogram power from 0 to 21 years old. (A-D) Representative frontal electroencephalogram median spectra in selected patients, aged 4 months, 4 years, 10 years, and 20 years, show that slow-
delta (0.1-4 Hz) oscillations are present at all ages during propofol general anesthesia maintenance. Alpha (8-13 Hz) oscillations are observed in patients after 1 year of age. (E-H) Corresponding spectrograms in selected patients during propofol general anesthesia maintenance show that slow-delta (0.1-4 Hz) oscillations are present in all patients, and that alpha (8-13 Hz) oscillations are observed in patients greater than 1 year of age. (I) Total electroencephalogram power (0.1-40 Hz) for each subject, plotted as a function of age (shown in circles). The central blue line represents a multiple linear regression (third degree polynomial) describing the relationship between total electroencephalogram power and age. The shaded bounds represent the 95% confidence interval for this regression model. The regression equation, F-statistic, and p-value are displayed. The adjusted $R^2 = 0.48$ for the model. Total electroencephalogram power increased with increasing age, peaking at approximately 8 years of age, then declining and plateauing during the adolescent years. Reprinted, with permission, from Lee et al. (2017), A Prospective Study of Age-Dependent Effects of Propofol on Frontal Electroencephalogram Dynamics in Children. Anesthesiology. 2017 Aug;127(2):293-306, Figure 2.
Figure 4. Median spectra and spectrograms in age groups. (A, E) Group 1 (4 months – 1 year). The median power spectrum and spectrogram show prominent power in the slow frequency band (0.1-1 Hz), and a broad secondary peak in power between 10 and 25 Hz. (B, F) Group 2 (>1-7 years). The median power spectrum and spectrogram show prominent power in the slow (0.1-1 Hz) and alpha (8-13 Hz) frequency bands. (C, G) Group 3 (>7-14 years). The median power spectrum and spectrogram show prominent power in the slow and alpha frequency bands. (D, H) Group 4 (>14-21 years). The median power spectrum and spectrogram show prominent power in the slow and alpha frequency bands. Statistically significant differences between age groups can be found in Table 2. Adapted, with permission, from Lee et al. (2017), A Prospective Study of Age-Dependent Effects of Propofol on Frontal Electroencephalogram Dynamics in Children. Anesthesiology. 2017 Aug;127(2):293-306, Figure 3.
Figure 5. Median coherence and coherograms in age groups. (A, E) Group 1 (4 months – 1 year). The median coherence and coherogram show faint slow (0.1-1 Hz) coherence, but no significant frontal coherence is observed overall. (B, F) Group 2 (>1-7 years). The median coherence and coherogram exhibit some slow coherence and significant alpha (8-13 Hz) coherence. (C, G) Group 3 (>7-14 years). The median coherence and coherogram show a relative increase in slow coherence, and strong alpha coherence. (D, H) Group 4 (>14-21 years). The median coherence and coherogram show a significant increase in slow coherence and a relative decrease in alpha coherence. Statistically significant differences between age groups can be found in Table 2. Adapted, with permission, from Lee et al. (2017), A Prospective Study of Age-Dependent Effects of Propofol on Frontal Electroencephalogram Dynamics in Children. Anesthesiology. 2017 Aug;127(2):293-306, Figure 4.
Figure 6. Age-related changes in slow and alpha electroencephalogram power. (A) A multiple linear regression model was used to describe the relationship between frontal slow (0.1-1 Hz) power and age (blue). (B) A multiple linear regression model was used to describe
the relationship between frontal alpha (8-13 Hz) power and age (red). The shaded bounds represent the 95% confidence intervals for these regression models. (C) Frontal slow power peaks at approximately 11.6 years of age, and alpha power peaks at approximately 7.3 years of age. The difference between these peak ages is statistically significant (95% confidence interval, 3.0 – 5.5 years). Alpha oscillation power was greater than slow power from 3.6 to 5.3 years of age, whereas slow power was greater than alpha power from 10.5 to 20.3 years of age (95% confidence interval, bootstrap analysis). Reprinted, with permission, from Lee et al. (2017), A Prospective Study of Age-Dependent Effects of Propofol on Frontal Electroencephalogram Dynamics in Children. Anesthesiology. 2017 Aug;127(2):293-306, Figure 5.
Figure 7. Age-related changes in slow and alpha coherence. (A) A multiple linear regression model was used to describe the relationship between frontal slow (0.1-1 Hz) coherence and age (blue). Slow coherence appeared to increase linearly between 1 and 21 years of age. (B) A
multiple linear regression model was used to describe the relationship between frontal alpha (8-13 Hz) coherence and age (red). Alpha coherence peaked at 8.9 years of age (95% confidence interval, 7.4-12.2 years). The shaded bounds represent the 95% confidence intervals for these regression models. (C) Alpha oscillation power was greater than slow power from 2.6 to 14 years of age (95% confidence interval, bootstrap analysis). The horizontal green line represents the ages for which there is a statistically significant difference between slow and alpha coherence. Reprinted, with permission, from Lee et al. (2017), A Prospective Study of Age-Dependent Effects of Propofol on Frontal Electroencephalogram Dynamics in Children. Anesthesiology. 2017 Aug;127(2):293-306, Figure 6.
Figure 8. Frontal electroencephalogram power and coherence in infants under 2 years of age. (A) Median power spectra for two groups of subjects are shown: subjects between 4
months and 1 year of age (n=4; blue) and subjects between 1 year and 2 years of age (n=3; red). The shaded bounds represent the 95% confidence intervals for these power spectra. The spectra show that slow (0.1-1 Hz) oscillations are consistently observed in all subjects. However, the power spectrum in subjects less than 2 years old shows the relative absence of well-defined alpha (8-13 Hz) oscillations, instead showing a higher frequency broad increase in electroencephalogram power for 12-25 Hz oscillations. Electroencephalogram power is significantly greater in the 1 – 2 year age group relative to the 4 month – 1 year age group for the following frequency ranges: 0 – 15.14 Hz and 20.51 – 33.69 Hz (95% confidence interval, bootstrap analysis). Horizontal green lines represent the frequency ranges for which there is a significant difference. (B) Median coherence in patients less than 2 years old shows the absence of alpha (8-13 Hz) oscillation coherence in subjects between 4 months and 1 year of age, with alpha oscillation coherence becoming apparent in subjects between 1 year and 2 years of age. Frontal coherence is significantly greater in the 1-2 year age group relative to the 4 month – 1 year age group over for the 6.35 – 11.72 Hz frequency range (95% confidence interval, bootstrap analysis). The horizontal green line represents the frequency ranges for which there is a significant difference. Reprinted, with permission, from Lee et al. (2017), A Prospective Study of Age-Dependent Effects of Propofol on Frontal Electroencephalogram Dynamics in Children. Anesthesiology. 2017 Aug;127(2):293-306, Figure 7.
Figure 9. Age-varying spectrogram and coherogram during propofol general anesthesia. 

(A) An age-varying spectrogram for patients 1-21 years old shows that the frontal electroencephalogram structure during propofol-induced unconsciousness appears to be qualitatively consistent across age and is comprised of slow (0.1-1 Hz) oscillations and alpha (8-13 Hz) oscillations. At the same time, the power of these oscillations changes as a function of age. Specifically, we observed age-dependent changes in the spectrogram, with high frequency power declining with increasing age and alpha oscillation power significantly decreasing by approximately 16 years of age. 

(B) An age-varying coherogram for patients 1-21 years old consistently shows prominent alpha oscillation coherence. In addition, slow oscillation coherence appears to increase with age, particularly after about 11 years of age. Adapted, with permission, from Lee et al. (2017), A Prospective Study of Age-Dependent Effects of Propofol on Frontal Electroencephalogram Dynamics in Children. Anesthesiology. 2017 Aug;127(2):293-306, Figure 8.
Figure 10. Patient Selection for Sevoflurane Analysis. We identified 192 eligible cases from children between 0 and 40 months of age scheduled for elective surgery. Of these, EEG data was collected from 106 children, and of these, we excluded 15 cases for technical failure, clinical event affecting ability to continue study, or isoflurane anesthesia administered. Ultimately, 91 EEGs were analyzed, and the number of subjects within each age group is specified above. Adapted, with permission, from Cornelissen et al. (2017), Electroencephalographic markers of brain development during sevoflurane anaesthesia in children aged 0 to 3 years old. Br J Anaesth. Forthcoming 2018, Supplementary Table 1.
Figure 11. Frontal EEG spectral power for frequencies from 0.1 to 40 Hz during maintenance of sevoflurane general anesthesia. (A) Frontal spectrogram for individual subjects. (B) Age-varying spectral changes in the frontal spectrogram. Regressions are shown for frontal peak band-power in the (C) slow (0.1 – 1 Hz), (D) delta (1 – 4 Hz), and (E) alpha (8 – 12 Hz) frequency bands. Delta oscillations exhibited a small, steady increase in power with age. Alpha oscillations increased in power during infancy, peaking at approximately 10 months and
remaining at a sustained level with age. The solid line represents a fourth-degree polynomial regression model describing the relationship between age and EEG power, with equation, adjusted $R^2$, and F-statistic written in inset; dashed lines represent the 95th confidence boundaries of the regression model. The F7 electrode is presented using nearest neighbour Laplacian referencing. Adapted, with permission, from Cornelissen et al. (2017), Electroencephalographic markers of brain development during sevoflurane anaesthesia in children aged 0 to 3 years old. Br J Anaesth. Forthcoming 2018, Figure 1. (See Appendix B1 for frontal EEG spectral properties during sevoflurane-induced general anesthesia across age groups.)
Figure 12. Topographic EEG maps of spectral power for distinct frequency bands during sevoflurane general anesthesia in children 0 to 3 yrs. Topographic EEG maps detailing group-averaged power for each EEG frequency band in infants aged (A) 0 - 3 months, (B) 4 - 6 months, (C) 7 - 9 months, (D) 10 - 14 months, and toddlers aged (E) 15 - 17 months, and (F) 18 - 40 months. Slow (0.1-1 Hz), delta (1-4 Hz) and theta (4-8 Hz) activity is distributed across the scalp in all age groups. Alpha (8-12 Hz) activity is present to a greater degree from around 4 months, and is largely regionalized to the frontal cortex from around 7 months of age. Beta (12-25 Hz) and gamma (25-40 Hz) oscillations remain negligible across all ages. Reprinted, with permission, from Cornelissen et al. (2017), Electroencephalographic markers of brain development during sevoflurane anaesthesia in children aged 0 to 3 years old. Br J Anaesth. Forthcoming 2018, Figure 2.
Figure 13. Frontal and occipital power distribution at slow, delta and alpha frequencies during sevoflurane general anesthesia in children from 0 to 3 yrs. Peak frontal and occipital EEG power in the (A) slow (0.1 – 1 Hz), (B) delta (1 – 4 Hz), and (C) alpha (8 – 12 Hz)
frequency bands for each subject are plotted as a function of age. Solid line represents fourth degree polynomial regression model describing the relationship between age and EEG power (blue line, frontal power; red line, occipital power; dashed lines, 95th percentile confidence boundaries); inset schematic indicates the location of the frontal and occipital channels. Differences in power between frontal and occipital channels in the (D) slow, (E) delta, and (F) alpha frequency bands, are presented with 95% CI from bootstrap analysis (pink line, 97.5th percentile; green line, 2.5th percentile). Slow and delta oscillations exhibit a small and significant increase in frontal power compared to occipital power from mid-late infancy until ~26 to 30 months of age. Alpha oscillations emerge around 3-4 months of age, and exhibit a significant and sustained frontal predominance of power that begins to emerge at 8 months of age, and peaks at ~15 to 20 months of age. These data indicate that adult-like patterns of activity are detectable from infancy. F3 and O1 electrodes presented using nearest neighbour Laplacian referencing. Adapted, with permission, from Cornelissen et al. (2017), Electroencephalographic markers of brain development during sevoflurane anaesthesia in children aged 0 to 3 years old. Br J Anaesth. Forthcoming 2018, Figure 3.
Figure 14. Frontal coherograms and coherence during sevoflurane general anesthesia in children from 0 to 3 yrs. Frontal EEG coherence power for frequencies from 0.1 to 40 Hz during a 5-min period of surgical anesthesia. (A) Frontal coherograms for individual subjects at 2, 3, 5, 9, 11, 16, 19 and 37 months of age. (B) Age-varying coherence changes in frontal coherogram. Frontal coherence as a function of age is shown in the (C) slow (0.1-1 Hz), (D) delta (1-4 Hz), and (E) alpha (8-12 Hz) frequency bands for each subject. Slow and delta coherent oscillations are present in subjects from birth until around 8 months old. Alpha
coherence appears to emerge around 10 months of age and increases over subsequent months, persisting to 3 years of age. Solid line represents a third or fourth-degree polynomial regression model describing the relationship between age and EEG power, with equation, adjusted R², and F-statistic written in inset; dashed lines represent the 95th percentile confidence boundaries of the regression model. Adapted, with permission, from Cornelissen et al. (2017), Electroencephalographic markers of brain development during sevoflurane anaesthesia in children aged 0 to 3 years old. Br J Anaesth. Forthcoming 2018, Figure 4. (See Appendix B2 for frontal coherence properties during sevoflurane-maintained general anesthesia across age groups.)
Figure 15. Global coherence during sevoflurane general anesthesia in children from 0 to 3 yrs. Topographic EEG maps detailing group-averaged coherence for each EEG frequency band in children aged (A) 0 - 3 months, (B) 4 - 6 months, (C) 7 - 9 months, (D) 10 - 14 months, (E) 15 - 17 months, and (F), and 18 - 40 months. Spatially coherent alpha (8-12 Hz) oscillations that are concentrated in the frontal channels emerge at 10-14 months of age. Global coherence is low across all remaining frequencies and locations in all ages during surgical anesthesia. This analysis suggests that global coherence becomes increasingly “adult-like” towards the end of the first year of life. Adapted, with permission, from Cornelissen et al. (2017), Electroencephalographic markers of brain development during sevoflurane anaesthesia in children aged 0 to 3 years old. Br J Anaesth. Forthcoming 2018, Figure 5.
Figure 16. Hypothetical model of age-varying changes in thalamocortical and cortico-thalamic connectivity during sevoflurane general anesthesia. (A) In adults, sevoflurane and propofol general anesthesia are characterized by anteriorization of alpha oscillations which are driven by frontal thalamocortical activity. (B) In infants 0 to 3 months of age, frontal alpha oscillations are absent and this is likely due to weak connectivity of projections from the thalamus to the frontal cortices and from the cortices to the thalamus. (C) In infants, from 4 to 9 months of age, alpha oscillations are observed and located over the frontal cortex. The
appearance of frontal alpha oscillations is thought to be driven by maturation of thalamocortical and cortico-thalamic connections. Alpha oscillations remain incoherent across the left and right frontal hemispheres at these ages and this may be due to immaturity of the corpus callosum, which serves to join the two hemispheres of the brain, and while in place from birth undergoes rapid maturation over the first 9 months of age. (D) Towards the end of infancy and into early childhood, children aged between 10 months and 3 years show highly synchronous and robust frontal alpha oscillations that mimic patterns of neural activity that are similar to that of the adult under sevoflurane or propofol general anesthesia and likely reflect highly functioning thalamocortical relay connectivity. Regions of interest shown in grey (adult), orange (0 to 3 months of age), green (4 to 9 months of age), and blue (10 months to 3 years of age).
Figure 17. Postnatal Brain Development in the Human. (A) Rate of synaptogenesis in the prefrontal cortex (black), language areas of the cortex (light blue), and sensorimotor cortex (dark blue). (B) White matter maturation (blue arrows indicate onset of mature myelination). Corpus callosum, thalamocortical and associated fibers are the last regions to myelinate. (C) Neurodevelopmental milestones, and (D) EEG features under sevoflurane maintenance anesthesia. Thick solid black lines represent age grouping in current study. Adapted, with permission, from Cornelissen et al. (2017), Electroencephalographic markers of brain development during sevoflurane anaesthesia in children aged 0 to 3 years old. Br J Anaesth. Forthcoming 2018, Figure 6. Figure originally adapted, with permission, from Thompson & Nelson (2001), Corel (1975), Dubois et al. (2014), and Frankenburg et al. (1992).
Figure 18. Case Illustration: Distinct Brain States Visible on EEG. This figure illustrates that distinct brain states are readily visible in the propofol-induced EEG in children, and reflects clinical level of anesthesia. Clinical events during this EEG recording are reported along the top of the spectrogram, e.g. “Start endoscopy”, “Patient begins moving”, “Propofol bolus”, etc. “Pattern 1” and “Pattern 2” indicate the start of the two brain states highlighted in this case. Pattern 1, shown roughly in the first half of this case, reflects a clinical state of light sedation, consistent with the higher frequency beta-gamma oscillations seen in the spectrogram. During this state, the patient began moving in response to a procedural stimulus, and a propofol bolus was given to deepen anesthesia, with corresponding transitions in both clinical state and EEG pattern. Pattern 2 shows the characteristic slow and alpha oscillations associated with propofol general anesthesia in children and adults. This case illustrates that the different brain states are readily apparent on the EEG and suggests that the spectrogram could be used to guide anesthetic management in children.
Appendix A1. Individual power spectra and spectrograms in infants under 1 year of age during propofol general anesthesia. Individual spectra (A-D) and spectrograms (E-H) for patients under 1 year of age show an absence of alpha oscillation power at 4 months old, but shows alpha power for the 5, 10, and 11 month old patients.
Appendix A2. Individual coherence and coherograms in infants under 1 year of age during propofol general anesthesia. Individual coherence plots (A-D) and coherograms (E-H) for patients under 1 year of age illustrate the lack of coherent alpha oscillations in this age group.
Appendix B

A  Frontal group-median spectrograms

B  Frontal group-median spectrum

Appendix B1. Frontal EEG spectral properties across age groups during sevoflurane general anesthesia. Frontal group-averaged (A) power spectrograms and (B) power spectra across all frequencies in children from 0 – 3, 4 – 6, 7 – 9, 10 - 14, 15 - 17 and 18 - 40 months of age. For power spectra: solid line, median; shaded area, 25th-75th percentile. F7 electrode presented using nearest neighbour Laplacian referencing.
Appendix B2. Frontal coherence properties across age groups during sevoflurane general anesthesia. Frontal group-averaged (A) coherograms and (B) frontal group-median coherence across all frequencies in children aged 0 - 3 months, 4 - 6 months, 7 - 9 months, 10 - 14 months, 15 - 17 months, and 18 - 40 months. Left frontal (F7-Fp1) to right frontal (F8-Fp2) coherence is shown. Frontal group-median coherence showed slow and delta oscillations were highly coherent in young infants but not in infants older than 8 months, while alpha oscillations
became coherent around 10 - 14 months of age. For coherence: solid line, median; shaded area, 25th-75th percentile. F7 electrode presented using nearest neighbor Laplacian referencing.