Changes in the Propofol-Induced Frontal Electroencephalogram in Children With Autism Spectrum Disorder

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aided in my orientation to the laboratory and submitting my first IRB; and finally, Sheri Leone, who makes everything in the group run like clockwork and has been one of my most treasured supports during the research year.
ABBREVIATIONS

5-HT = serotonin
Ach = acetylcholine
ASD = autism spectrum disorder
BIS = bispectral index
CNS = central nervous system
DA = dopamine
dB = decibels
DR = dorsal raphe
EEG = electroencephalogram
E/I = excitatory/inhibitory
GA = general anesthesia
GABA = gamma aminobutyric acid
Gal = galanin
His = histamine
HPD = highest posterior density
hr = hour(s)
LC = locus coeruleus
LDT = laterodorsal tegmental area
LH = lateral hypothalamus
KCC2 = potassium-chloride co-transporter 2
m = minute(s)
MRI = magnetic resonance imaging
n = number of subjects
NE = norepinephrine
NKCC1 = sodium-(potassium)-chloride co-transporter 1
NMDA = N-methyl-D-aspartate
N₂O = nitrous oxide
NT = neurotypical
OR = operating room
POA = preoptic area
POD = post-operative delirium
PPT = pedunculopontine tegmental area
PSI = patient state index
PV+ = parvalbumin-positive
s = second(s)
SMD = standardized mean difference
TMN = tuberomamillary nucleus
Vpag = ventral periaqueductal gray
yr = year(s) old
ABSTRACT

General anesthetic drugs induce characteristic oscillations within brain circuits that can be readily assessed using the electroencephalogram (EEG), allowing a controlled and noninvasive way to study the circuitry of the human brain. During propofol-induced unconsciousness, frontal EEG demonstrates large-amplitude slow-delta oscillations (0.1-4 Hz) and frontally coherent alpha oscillations (8-13 Hz), the latter of which are thought to reflect propofol’s actions within frontal thalamocortical circuits. Previous studies from our laboratory show significant age-related changes in the propofol-induced frontal EEG that are thought to reflect the development of underlying circuits mediated by GABAergic interneurons. Interestingly, autism spectrum disorder (ASD) is increasingly seen in the clinical setting and is thought to arise from deficits in GABAergic signaling leading to abnormal neurodevelopment and neuromodulation. To investigate differences in how ASD patients respond to the GABAergic agent propofol, we recorded continuous 4-channel frontal EEG during the routine care of ASD patients who received propofol as a primary anesthetic for endoscopy and compared the data to a similar cohort of neurotypical (NT) patients. We found that the trajectory of age-dependent alpha (8-13 Hz) and slow (0.1-1 Hz) power showed notable differences in ASD vs. NT patients. Additionally, we found that the incidence of burst suppression or prolonged suppression was significantly higher in ASD vs. NT patients (28.57% vs. 12.30%, p<0.01) despite lower propofol doses. These results suggest that ASD patients respond differently to the GABAergic drug propofol and may have lowered anesthesia requirements for GABAergic agents compared to NT patients. These differences and their age-dependence may reflect underlying differences in GABAergic circuit function and development in ASD. As a similar pattern of decreased alpha power and increased sensitivity to burst suppression develops progressively with age in NT adults, our results may signify a form of accelerated neuronal aging in adolescent ASD patients. Taken together, our results suggest that measuring the propofol-induced EEG in ASD patients may enable insights into the underlying differences in neural circuitry of ASD and yield safer practices for managing anesthesia for ASD patients.
INTRODUCTION

Overview of General Anesthesia

General anesthesia (GA) is defined as a drug-induced, reversible condition characterized by unconsciousness, akinesia, analgesia, and amnesia while maintaining physiological homeostasis. A distinct condition from sleep, GA is more consistent with a reversible drug-induced coma. It can be induced by a number of agents, the majority of which are thought to act at a molecular level by influencing gamma aminobutyric acid (GABA) or N-methyl-D-aspartate (NDMA) signaling in the central nervous system (CNS). After GA was first harnessed for medical use in the 19th century, it is estimated that 21 million patients per year receive GA for surgery in the United States alone.

For decades, the exact mechanism by which general anesthetics produced unconsciousness was considered a “black box” that was impossible to solve. However, recent advances in the understanding of the molecular and neural circuit mechanisms of various anesthetics have brought us closer to establishing a neurophysiological definition of GA.

Molecular and Neural Circuit Mechanisms of Propofol

Propofol (2,6 di-isoprophylphenol) is a short-acting sedative-hypnotic agent that is one of the most common agents used for both sedation and GA. It has been found to bind postsynaptically to the second transmembrane domain of the β3 subunit of the GABA \(_A\) receptor. It acts as an allosteric agonist and potentiates GABA \(_A\) activity by slowing chloride channel-closing time, thus allowing greater hyperpolarization of the post-synaptic neuron and greater downstream inhibition.

Propofol is formulated as an oil-in-water emulsion for intravenous use, which gives it its characteristic milky-white appearance. It is highly lipophilic and rapidly crosses the blood-brain barrier to result in rapid onset of action (15-45s). Emergence is similarly rapid (10-15m if infused for <3hr) because propofol is rapidly redistributed from the brain into peripheral tissues and undergoes metabolic clearance.
Propofol acts on multiple sites to induce global CNS inhibition, as GABAergic interneurons are widely dispersed throughout the cortex, thalamus, and brainstem with differing effects depending on the region (Figure 1). In the cortex, propofol enhances GABA-mediated inhibition of the excitatory pyramidal neurons. In the thalamus, propofol increases GABA-mediated inhibition of the thalamic reticular nucleus, which decreases excitatory input from the thalamus to the cortex. In the brainstem, propofol promotes GABA-mediated inhibition from the preoptic area (POA) to the cholinergic, monoaminergic, and orexinergic arousal centers in the brainstem, which also influence cortical activity. Ultimately, decreasing excitatory inputs from the thalamus and the brainstem to the cortex further enhance inhibition of the cortical pyramidal neurons.

**Figure 1. Neurophysiological actions of propofol in the CNS.** Propofol enhances GABAergic transmission in the cortex and at the inhibitory projections from the POA to the arousal centers.10

Abbreviations: 5HT, serotonin; Ach, acetylcholine; DA, dopamine; DR, dorsal raphe; GABA, gamma aminobutyric acid; Gal, galanin; His, histamine; LC, locus coeruleus; LDT, laterodorsal tegmental area; LH, lateral hypothalamus; NE, norepinephrine; POA, preoptic area; PPT, pedunculopontine tegmental area; TMN, tuberomammillary nucleus; Vpag, ventral periaqueductal gray.
Use of the Electroencephalogram in GA and Brain Monitoring

Communication in the CNS consists of coordinated action potentials (known as spikes) between neurons, which lead to extracellular electrical potentials referred to as local field potentials (Supplementary Figure 2). The summation of these local field potentials produced by neurons can be readily measured by placing electrodes on the scalp in a technique called electroencephalography (EEG). Placement of the electrodes and the underlying structure of the brain influence the strength of the signal. For example, oscillatory local field potentials produced in the cortex are readily measured at the scalp by the electroencephalogram (EEG), as the geometry of the excitatory pyramidal neurons favors production of large local field potentials. Meanwhile, subcortical structures (i.e., thalamus) produce more modest local field potentials that are technically difficult to directly detect at the scalp, but their rich interconnection with and influence on cortical structures implies that the scalp EEG reflects the activity of both cortical and subcortical structures.

In 1937, it was first reported that all general anesthetic drugs induce highly structured oscillations within brain circuits and networks that are readily observed in the EEG, a finding that multiple subsequent studies would confirm. The functional significance of these EEG oscillations was unclear for many decades, but there is now substantial evidence that these oscillations comprise a fundamental mechanism by which anesthetic drugs induce altered states of arousal and unconsciousness. Neural oscillations provide a means to synchronize and coordinate activity within and between brain circuits and networks. General anesthetic drugs appear to induce non-physiologic oscillations that override or disrupt this coordinated activity, such that the presence of these overriding oscillations is closely associated with the depth of patients’ states of unconsciousness.

Our laboratory has pioneered the use of the anesthesia-induced EEG to characterize systems-level mechanisms of general anesthetic drugs. There is growing evidence that anesthesia-induced EEG oscillations are intimately related to their underlying molecular pharmacological and neural circuit-level mechanisms of action (e.g., GABA, NMDA, α2 adrenergic). Unconsciousness induced by the
GABAergic anesthetics propofol and sevoflurane is characterized by the presence of slow (0.1-1 Hz) and frontal alpha (8-13 Hz) oscillations in the EEG (Figure 2).\(^9,20,21\)

Propofol- and sevoflurane-induced slow oscillations reflect periods of cortical neuronal silence, punctuated by brief periods of firing that are asynchronous or “fragmented” across different cortical areas.\(^{20,26}\)

The mechanisms underlying these anesthesia-induced slow oscillations are not fully understood, but they may reflect decreased ascending arousal signaling from the brainstem and increased cortical hyperpolarization, which are both mediated by increased GABA inhibition. Propofol- and sevoflurane-induced frontal alpha oscillations appear to have a GABA-mediated thalamocortical mechanism in which anesthesia-enhanced GABA activity amplifies inhibitory synapses, effectively slowing the dynamics of the circuit in such a way that the neurons fire in a ~10 Hz pattern as opposed to their usual, asynchronous pattern.\(^{14,27,28}\)

**Figure 2. The clinical neurophysiology of propofol.** Propofol produces stereotyped alpha (8-13 Hz) and slow (0.1-1 Hz) oscillations that can be visualized as the EEG spectrogram (upper right). Alpha waves reflect oscillatory activity within thalamocortical circuits, and slow oscillations reflect cortical OFF states where neurons are unable to fire.\(^{14,17,20,21}\)
Unfortunately, despite these insights, the use of the unprocessed EEG and spectrogram to monitor brain states under GA and sedation is not yet standard practice in anesthesiology. The majority of institutions instead use a variety of depth-of-anesthesia monitors that produce a numerical patient state index (PSI) from 0 (no brain activity) to 100 (fully awake). These indices were developed by recording the EEG and behavioral endpoints after exposure to increasing doses of specific general anesthetics (usually propofol or volatile agents) in a healthy adult patient cohort; the algorithms are often proprietary. However, the use of these monitors has not been shown to prevent intraoperative awareness as intended. Furthermore, due to the conditions of their development, they are not applicable for certain patient cohorts (e.g., pediatric patients) and anesthetics such as ketamine, dexmedetomidine, and nitrous oxide (N₂O). Due to these limitations, our laboratory has advocated for training anesthesiologists to recognize and interpret the brain states defined by drug-specific EEG signatures found in the unprocessed EEG and spectrogram, rather than an arbitrary number calculated from a proprietary algorithm.

**Overview of Pediatric Neurodevelopment**

The pediatric brain under anesthesia represents one such case where typical depth-of-anesthesia monitors do not apply. This is due to dynamic changes in the same underlying circuitry assessed by EEG.

Development of the brain begins at the fifth week of gestation but remains incomplete at the time of birth, with maturation continuing through childhood and adolescence. After age 25, only modest changes in organization occur (Figure 3).
Proper neurodevelopment relies on the flawless execution of processes such as neurogenesis, apoptosis, myelination, axonal and dendritic growth, synaptogenesis, and synaptic pruning to correctly organize a dizzying number of neurons and support cells throughout the CNS. In humans, synaptogenesis begins at birth and undergoes a period of rapid expansion that culminates in maximum synaptic density by approximately 2 yr. Synaptic density decreases gradually thereafter via synaptic pruning, which accelerates during adolescence with an estimated 40-50% of total synaptic density lost from onset of puberty to adulthood. In parallel, the strength of excitatory synapses appears to increase from birth to approximately 5 yr, and then gradually declines to a plateau at approximately 18 yr. The absolute volume of cortical grey matter also declines in late childhood and adolescence, leading to cortical thinning from “back to front” with the frontal regions controlling executive functions as the last to mature. Ultimately, the majority of neurons and their synaptic connections will ultimately be pruned. It is thought that this process ensures that appropriate connectivity is established.
Anesthesia-Induced EEG in Development and Aging

The time course of typical human brain development is reflected in both the awake and anesthesia-induced EEG. In the awake state, the pediatric EEG has been shown to evolve with age in a fashion that is likely reflecting underlying changes in brain circuitry. In general, there is a gradual increase in the frequency of background activity and the development of an occipital alpha wave following the development of thalamocortical circuits between four months and one year of age.

Similarly, our laboratory recently demonstrated that the anesthesia-induced EEG shows striking developmental changes in pediatric patients. Frontal alpha waves induced by propofol or sevoflurane are absent initially in very young children, but develop during the first year of life, along a time course that is consistent with the functional development of thalamocortical connections (Figure 4). In older children, anesthesia-induced EEG power significantly increases from infancy through approximately 6 years of age, subsequently declining to a plateau at approximately 21 years (Figure 5).

Figure 4. Global and frontal coherence in young children. (A) Studies of sevoflurane-induced EEG in infants < 6 months of age show that they do not have coherent frontal alpha waves. (B) Analysis of sevoflurane-induced EEG in children from 0 through 1.5 years show that frontal alpha waves become coherent at approximately 1 year of age.
Figure 5. Relationship of total EEG power (0.1-40 Hz) with age in the propofol-induced frontal EEG in children aged 0 to 21 yr.\textsuperscript{50}

A similar study identified significant age-related changes in the EEG of elderly neurotypical patients, showing decreased alpha (8-13 Hz) power and increased incidence of burst suppression.\textsuperscript{52} Burst suppression is a state of profound brain inactivation, easily visible on the EEG, that is characterized by epochs of low amplitude activity or isoelectricity corresponding to electrocortical silence (“suppression”), interrupted by epochs of relative high amplitude activity (“bursts”) (Figure 6).\textsuperscript{53} Together, these results were thought to reflect neurobiological and neuroanatomical changes with aging.
Our laboratory hypothesized that these age-related changes in anesthesia-induced EEG power could reflect the state of underlying GABAergic circuits. Parvalbumin-positive (PV+) GABAergic interneurons are known to regulate the onset and time course of critical periods of neurodevelopment. During early development, synaptic proliferation involving cortical pyramidal cells could result in a greater degree of synchronous activity under anesthesia, and thus greater amplitude of post-synaptic potentials and signal on EEG. Later in development, synaptic pruning could in turn reduce the amplitude of anesthesia-induced postsynaptic currents and EEG (Figure 7). This hypothesis that age-dependent changes in brain development are reflected in the anesthesia-induced EEG is supported by similar findings in studies of orphans, in which resting alpha EEG power was shown to be significantly reduced after early childhood neglect implying stunted neurodevelopment. At the other end of the spectrum in the aging population, it is hypothesized the observed changes reflected progressive cortical thinning, particularly involving the thalamocortical circuits responsible for generation of the alpha oscillation. Taken together, these results suggested that the paradigm of the anesthesia-induced EEG could be harnessed to
non-invasively characterize the state of underlying GABAergic circuits not only in normal development and aging, but also in disorders of neurodevelopment such as autism spectrum disorder.

Figure 7. The relationship between age and EEG power reflects synaptogenesis and synaptic pruning. Synaptogenesis, which reaches a peak around 6 years of age, results in greater postsynaptic currents and thus a higher total EEG power. Conversely, synaptic pruning results in lessened postsynaptic currents and thus a lower total EEG power.

Overview of Autism Spectrum Disorder (ASD)

ASD is a neurodevelopmental disorder characterized by deficits in social interaction and restricted, repetitive patterns of behavior, interests, and activities. Patients with ASD often display hypersensitivity to visual, tactile, and auditory stimuli, and hyposensitivity to pain and temperature stimuli. ASD was first described in the literature in 1943 by Leo Kanner, although it is likely that Hans Asperger was the first to identify a cohort of patients with the disorder. Beyond its core features, ASD is a heterogeneous condition that ranges in severity from mild impairment to profound disturbances in behavior and communication. Although once considered rare, ASD
affected nearly 1 in 68 children in the U.S. in 2010, with a prevalence that continues to rise. Of note, this rising prevalence may actually reflect broader definitions of ASD, improved diagnostic techniques, and/or increased awareness of core symptoms. Risk factors include male gender (4:1 predominance), certain genetic mutations and syndromes such as tuberous sclerosis complex (TSC) and Rett syndrome (MECP2 mutation), and maternal advanced age, infection, or autoimmune disease. Additionally, there is evidence of high heritability with a 36-98% concordance rate in monozygotic twins and a variably increased risk of ASD if a child has affected siblings. Symptoms tend to appear early, often by 12 to 18 months of age, and can manifest as a regression from seemingly normal development. There is no prevention, standard treatment, or cure for ASD. There is also no specific biomarker or neuroanatomical abnormality that defines ASD, and thus diagnosis is based on standardized behavioral assessments.

**Pathophysiology of ASD**

Theories regarding the pathophysiology of ASD have greatly evolved from the original “refrigerator mother” hypothesis, wherein children developed ASD due to cold, unfeeling mothers. However, the pathophysiology of ASD remains hotly debated. Genetic factors are clearly involved, but all known genetic defects associated with ASD together account for only a small fraction of cases. Furthermore, a purely genetic cause does not explain the wide heterogeneity in clinical presentation, discordant development in monozygotic twins, and families with occurrence of both ASD and “ASD-like” traits. One harmonious explanation is that inherent genetic susceptibility must be combined with an environmental insult in order to interrupt typical neurodevelopment. As exact genetic susceptibilities and environmental exposures may vary, this may also account for the known heterogeneity in the ASD phenotype. Recent evidence suggests that for many patients, this incident may occur in utero, with deficits in neuronal proliferation, differentiation, migration, and synaptic refinement leading to dysfunctional neuronal circuits.

Excitatory/inhibitory (E/I) imbalance is thought to lie at the heart of ASD. Clinically, this is supported by the high comorbidity of epilepsy in ASD patients, with
estimates ranging from 5 to 44%. In Rett’s syndrome, the risk of epilepsy is estimated to be as high as 94%. It is further estimated that 60% of ASD patients lacking clinical epilepsy display epileptiform activity on sleep EEG, suggesting subclinical E/I imbalance. Additionally, as inhibition is known to mediate critical periods of neurodevelopment in which the response to sensory stimuli is “sharpened,” it is theorized that reduced inhibitory influence could lead to excessive “noise” predisposing to sensory abnormalities as often seen in ASD. However, any defect in E/I balance (including hypoexcitability) could interfere with neurodevelopment and signal propagation throughout the brain.

Several published studies suggest that a loss of inhibitory GABA signaling contributes to the pathogenesis of the E/I imbalance in ASD. For example, the typical developmental shift of GABA from an excitatory to inhibitory neurotransmitter depends on sequential maturation of the bumetanide-sensitive sodium-(potassium)-chloride co-transporter 1 (NKCC1) to potassium-chloride co-transporter 2 (KCC2). ASD patients have been shown to have an increased NKCC1:KCC2 ratio, favoring excitability, and administration of bumetanide has been shown to reduce ASD behavior in both human patients and animal models of ASD. Genetic studies of human patients have revealed evidence of linkage disequilibrium in GABA receptor subunits and primary expression of ASD-related genes in GABAergic interneurons. In vivo as well as in vitro post-mortem studies of ASD patients have variably shown reduced levels of GABA-synthesizing enzymes, GABA receptors, and GABA itself throughout the brain. This is echoed in numerous animal models of ASD. Finally, a recent study demonstrated through magnetic resonance spectroscopy that there was no correlation between GABA levels and performance on a GABA-dependent sensory task in ASD patients, whereas increasing GABA was significantly linked to greater success in control patients.

**Structural Changes in ASD**

Given the spectrum of potential causal factors described above, it is unsurprising that characterizing the structural differences that occur in the brains of patients with ASD has been similarly elusive. This is partially because results vary
widely depending on the study design and are often limited by small sample size, but it is doubtless that the inherent spectrum of the disorder plays a role in the difficulty of identifying a precise phenotype. Nevertheless, the past few decades have shed new light on the most common findings in ASD patients.

GABAergic interneurons govern the process of synaptic refinement, which acts to prune away unnecessary connections and strengthen necessary ones. Defects in cell adhesion molecules such as SH3 and multiple ANKyrin repeat domains (Shank), which mediate synapse formation and dendritic spine maturation, have been associated with ASD.\textsuperscript{98} Post-mortem analyses of children and adolescents with ASD demonstrate abnormally high synaptic densities in multiple areas of the brain, thought to emerge from a defect in net spine pruning (16\% vs. 41\% decline).\textsuperscript{99}

Interestingly, children with ASD have been found to experience accelerated head growth during infancy and increased brain size (2-10\%) that may grossly reflect this failure of synaptic pruning.\textsuperscript{100-103} In particular, localized expansion of the frontal cortex is observed in early ASD. It is thought to involve both grey and white matter,\textsuperscript{104,105} and could indicate a mixture of over-exuberant synapse formation and failure to prune. However, this initial overgrowth is followed by a progressive, marked reduction in brain size by young adulthood associated with microglial activation that may suggest age-related neurodegeneration.\textsuperscript{106} A longitudinal study of cortical thickness in male ASD patients found evidence of accelerated expansion in early childhood, accelerated thinning in later childhood and adolescence, and decelerated thinning in early adulthood.\textsuperscript{107} This predominantly affected the frontal, parietal, and occipital regions. Interestingly, adolescence and young adulthood is often a time when cognitive and behavioral functioning plateaus or deteriorates in ASD. Altered cortical thickness has been shown in other studies to be the most accurate marker for ASD in brain anatomy imaging.\textsuperscript{108}

To our knowledge, the anesthesia-induced EEG has not been systematically used to investigate underlying neuronal circuit changes in ASD. Numerous EEG studies of ASD exist, including sleep EEG, but the heterogeneity of the methodology used has resulted in literature that is inconsistent and often contradictory. Conversely, general
Anesthetics such as propofol induce predictable and highly structured oscillations through its action on GABAergic circuits, which subsequently lead to interruption of physiological oscillations and altered consciousness. The high signal-to-noise ratio of these characteristic oscillations allow us to more precisely identify the state of GABAergic interneurons in ASD patients compared to prior studies.

**Anesthetic Management of Pediatric Patients with ASD**

Children with ASD are known to have an increased rate of overall hospital contact compared to those without ASD. Due to this as well as the rising prevalence of ASD, it is increasingly common to encounter pediatric patients with ASD in the operating room (OR) setting. In particular, an unusually high number of pediatric patients with ASD present to the hospital for endoscopic procedures (e.g., esophagogastrroduodenoscopy (EGD), colonoscopy, or sigmoidoscopy, anal manometry, rectal Botox). This is because gastrointestinal (GI) complaints are extremely common in ASD; the reported prevalence of any gastrointestinal (GI) disorders in ASD ranges from 9% to 91%, with constipation being the most common complaint.

Due to the aforementioned hypersensitivity to sensory stimuli, children with ASD often require sedation and general anesthesia for even minor procedures. Clinicians tend to give premedication with oral midazolam or ketamine to patients with ASD, but there is little guidance for how to titrate the anesthetic management of pediatric patients with ASD despite the known differences in their neurodevelopment. If we identify increased risk of burst suppression, either due to endogenous dysfunction or higher medication doses, this could indicate that further research is necessary to identify the appropriate dosing of sedative agents in children with ASD.

**Hypothesis**

Recent research into the use of anesthesia-induced EEG has allowed us to develop a paradigm of normal brain behavior during GA. Furthermore, the anesthesia-induced EEG offers a rare window into the developing brain, particularly the state of networks mediated by GABAergic interneurons. To investigate differences in how ASD
patients respond to the GABAergic agent propofol, we compared the propofol-induced frontal EEG in pediatric ASD patients vs. neurotypical (NT) patients. We also investigated the relationship of EEG power with age and the incidence in burst suppression in ASD vs. NT patients. The hypotheses were twofold: (1) underlying differences in GABAergic signaling in ASD lead to significant changes in the power spectra of the age-dependent propofol-induced EEG; and (2) these same circuit abnormalities predispose to increased susceptibility to burst suppression in ASD.
SPECIFIC AIMS

Aim 1. To characterize the propofol-induced frontal EEG in ASD pediatric patients.
   1.1. To characterize changes in power of slow, theta, alpha, beta, and gamma oscillations by age in ASD pediatric patients.
   1.2. To characterize changes in coherence of slow, theta, alpha, beta, and gamma oscillations by age in ASD pediatric patients.

Aim 2. To investigate the changes in the propofol-induced frontal EEG power in ASD vs. NT pediatric patients.
   2.1. To develop a model accounting for differences in clinical characteristics between ASD and NT cohorts.
   2.2. To quantify changes in the amplitude of alpha (8-13 Hz) oscillations by age in ASD vs. NT pediatric patients.
   2.3 To quantify changes in the amplitude of slow (0.1-1 Hz) oscillations by age in ASD vs. NT pediatric patients.

Aim 3. To characterize the incidence of burst suppression in the propofol-induced frontal EEG in ASD vs. NT pediatric patients.
   3.1. To identify epochs of burst suppression in the propofol-induced frontal EEG in ASD and NT pediatric patients.
   3.2. To compare baseline characteristics and total medication doses received prior to the onset of burst suppression in ASD vs. NT pediatric patients.
   3.3. To compare the incidence of burst suppression in ASD vs. NT pediatric patients.
MATERIALS AND METHODS

Location

All data was collected in the Pediatric Endoscopy Center at the Massachusetts General Hospital for Children. The center manages a wide age range of patients, from neonates to young adults. The providers also see a disproportionate number of ASD patients due to its affiliation with the Lurie Center for Autism. It is a fast-paced and high-volume clinical environment with a relatively standardized anesthetic approach, in which most patients receive propofol infusions for maintenance anesthesia.

Subject Selection and Data Collection

The Human Research Committee at the Massachusetts General Hospital (MGH) approved this prospective observational study. Patients with previously diagnosed ASD were identified using the DSM-V definition, which encompasses prior diagnoses of autism, Asperger’s syndrome, and pervasive developmental disorder not otherwise specified (PDD-NOS). As DSM-V further specifies that the earliest age for diagnosis of ASD is 2 yr, only patients aged greater than 2 yr were investigated. NT patients were defined as those lacking neurological or psychiatric abnormalities, including ASD, attention deficit hyperactivity disorders, seizures, and other congenital or psychiatric conditions.

Continuous 4-channel frontal EEG was recorded using the SEDLine monitor (Masimo Corporation, Irvine, CA) during routine anesthetic care of 88 pediatric patients with ASD aged 2 to 30 yr between July 1, 2015 and May 1, 2016. Two other members of our laboratory, Johanna M. Lee and Kristina Terzakis, had previously recorded continuous 4-channel frontal EEG using the SEDLine monitor during the routine anesthetic care of 157 NT patients aged 2 to 30 yr between July 1, 2014 and July 1, 2015. All data were recorded without experimental intervention, and anesthetic dosing was decided strictly by the anesthesiologist. All data were stored in a de-identified form. EEG data were recorded with a pre-amplifier bandwidth of 0.5-92 Hz, sampling rate of 250 Hz, and a 16-bit, 29 nV resolution. The SEDLine Sedtrace electrode array records from electrodes located approximately at positions Fp1, Fp2, F7, and F8, with
the ground electrode at Fpz and reference electrode at ~1cm above Fpz (Figure 8). Electrode impedance was less than 5 kΩ for each electrode.

Figure 8. Model patient with the SEDLine monitor correctly applied.115

From the ASD cohort, patients were excluded who did not receive propofol as their primary anesthetic agent (n=1), known neuroanatomical defects (n=4), lost diagnosis of autism (n=1), electroencephalogram artifacts or insufficient case duration (n=6), burst suppression or pre-burst suppression state for the entire case (n=12), and age greater than 23yr (n=2). Only patients between the ages of 2 and 23 yr were selected for the power analysis due to the scarcity of patients >23 yr in the data set. Epilepsy was ultimately not chosen as an exclusion criterion, as epileptiform EEG patterns in ASD patients commonly exist even without clinically diagnosed epilepsy.116 Patients with antiepileptic, anxiolytic, and antipsychotic medications were also not excluded as these are often held the morning of the procedure and are taken by the majority of patients. Ultimately, 42 patients with ASD were deemed suitable for spectral analysis.

From the NT cohort, cases were excluded for neurological or psychiatric comorbidities (n=37), electroencephalogram artifacts or burst suppression (n=14), non-propofol anesthetic (n=5), and ages <2 yr (n=10) or >23 yr (n=2). This resulted in a total cohort of 110 NT patients deemed suitable for spectral and coherence analysis.

Figure 9 summarizes the procedure of patient selection for each cohort.
Figure 9. Schematic of patient selection procedure from ASD and NT cohorts.

Data Processing

The EEG data was converted from its native .phy format to .edf readable data using a converter script and reformatted using the data editor Polyman. The modified .edf data was subsequently imported into Matlab (MathWorks, Natick, Massachusetts) where it was translated into a structure with five rows of data for each of the channels with a variable number of columns, depending on the data length, and a structure containing all the header information.

EEG data segments were selected using information from both the electronic anesthesia record (Metavision, Dedham, MA) and the EEG spectrogram. The concentrations of inhaled agents were automatically captured, and the concentrations of all other agents were manually recorded in the electronic anesthesia record by the
anesthesia providers. For each case, a 120-second epoch was identified with a stable propofol infusion rate and no other anesthetic drugs given for at least five minutes prior to the epoch. In cases with mask induction, it was specified that epochs must occur ≥ 5 minutes after the discontinuation of inhaled anesthetic (sevoflurane ± N₂O). Two lab members (E.C.W., P.L.P. for ASD data; J.M.L, K.T. for NT data) visually inspected EEG data for each subject and manually selected data segments meeting the above criteria as well as being free of artifacts, other noise, and presence of either burst suppression or emergence.

**Spectral Analysis**

The EEG power spectrum and spectrogram was computed for each subject using multitaper spectral methods enabled by the Chronux toolbox, summarized in Supplementary Figure 2.

The EEG power spectrum reports the energy in the EEG at a given frequency over time, and is calculated from an EEG derivation using equally weighted signals obtained from Fp1, Fp2, F7, and F8. Briefly, the four data channels are transformed into a Laplacian average of the data using the equation:

$$x_t^{LP} = x_t - \frac{1}{4} \sum_{j=1}^{0} x_j, \quad \text{where } j \neq t$$

This takes equal contribution of each of the paired right- and left-sided channels, and averages out potential independent noise from each channel. The EEG spectrum is then calculated by applying the discrete-time Fourier transform to the time-series data (mV over time).

The EEG spectrogram, which represents the frequency distribution of power over the 120-second analysis window, is estimated using a multitaper method. In this method, a data sequence x(t) is multiplied by a set of orthogonal sequences (tapers) to form a number of single taper periodograms which are then averaged as an estimate of the spectral density. We applied the following parameters: spectral resolution 3 Hz, sampling rate Fs 250 Hz, time-bandwidth product TW = 3, and window length T = 2-seconds with 0-second overlap, number of tapers K = 5. The frequency range was 0 to 40 Hz, which captures all 6 canonical EEG frequency bands (0.1-1 Hz slow waves, 1-4
Hz delta, 4-8 Hz theta, 8-13 Hz alpha, 13-25 Hz beta, and 25-40 Hz gamma). For each patient, the spectrum was calculated by using the median value for each frequency within the 120-second epoch. Average power within each spectral band was calculated from the patient’s spectrum and was converted to a decibel (dB) scale using:

$$Power(dB) = 10 \log_{10} Power$$

Additionally, an age-varying spectrogram was calculated using an overlapping (0.5 yr) sliding window spanning +/- 2 yr at each age value from 2 to 23 yr. This was performed by computing the median spectrum across patients in 0.5 yr age bins to illustrate the evolution of the spectrogram with increasing age. Finally, group level spectrograms and spectra were computed within the age groups defined above.

**Burst Suppression and Prolonged Suppression Analysis**

To characterize the incidence of burst suppression or prolonged suppression in ASD vs. NT patients, the original cohort was expanded to include the 10 cases noted to be in burst suppression or a pre-burst suppression state, as well as the 2 patients aged greater than 23 yr, for a total of 56 ASD patients. These were compared to a total cohort of 123 NT patients, which included 11 cases noted to be in burst suppression or a pre-burst suppression state, as well as 2 patients aged greater than 23 yr.

Each record was visually scored for episodes of burst suppression or prolonged suppression occurring at any time during the case. Burst suppression was defined operationally as the presence of at least three consecutive suppression events within a 1 min period. Prolonged suppression was defined operationally as the presence of a single suppression event lasting greater than 10 seconds. Patients showing either burst suppression or prolonged suppression at any point during the case were graded with a ‘1,’ whereas patients who did not show either event were graded with a ‘0.’ Two separate lab members (E.C.W., P.L.P.) reviewed all records and only those cases that were in agreement maintained a grade of ‘1.’ The time at the beginning of the first episode of burst suppression or prolonged suppression as well as the total duration (s) of burst suppression or prolonged suppression was determined for all confirmed cases.
To characterize the clinical characteristics associated with each confirmed case of burst suppression, the electronic medical record was accessed to provide relevant demographic data as well as case details including amounts of all medications administered.

**Statistical Analysis**

The primary outcome was changes in the structure of propofol-induced alpha (8-13 Hz) and slow (0.1-1 Hz) oscillations in based on analysis of power and coherence. We sought to characterize these changes both within the ASD cohort with age as well as for ASD vs. NT patients. The post-hoc analysis was incidence of burst suppression. The primary risk factor of interest for both investigations was a prior diagnosis of ASD as defined by DSM-V criteria.

Continuous variables that were normally distributed were presented as mean (SD) and categorical variables are presented as frequency counts (%). Preoperative characteristics between the ASD vs. NT patients were compared using standardized mean differences (SMD). A SMD greater than 0.2 (corresponding to a 15% non-overlap in the two distributions) was considered to be notable.

Since the inferences from a Bayesian analysis are richer and more informative than the traditional frequentist approach, the data were assessed using Markov Chain Monte Carlo techniques. Results were based on 10,000 iterations after a burn-in period of 1,000 iterations with thin set to 1. Two separate models were utilized to examine alpha and slow waves. The primary linear regression models assume no prior probabilities and analyzes differences in power between ASD and NT groups while adjusting for age, gender, propofol infusion rate (mcg/kg/min), propofol bolus (mg/kg), midazolam (mg/kg), fentanyl (mg/kg), and comorbidity of epilepsy. The optimal representation of age was determined to be age$^3$ according to the Bayes factor criteria. The 50% posterior probability and corresponding 80% two-sided posterior density intervals of the slope were reported. No a priori statistical power calculation was performed. All analyses were conducted using R statistical software (RStudio, version 3.2.2; R Foundation for Statistical Computing, Vienne, Austria).
For the ASD-only EEG 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 ASD age groups. We used a bootstrap procedure to calculate 95% confidence intervals for each spectral and coherence estimate, as well as for differences between spectra or coherence estimates. Briefly, we drew bootstrap samples (n=2000) from the median spectrum, median coherence, and differences in spectrum or coherence from each group. Bootstrap confidence intervals were calculated using the percentile method. To account for the spectral resolution of the spectral and coherence estimates, we defined differences as significant only if the significance threshold (95% confidence interval not containing zero) was met for contiguous frequencies over a frequency band equal to or exceeding the spectral resolution (2W). For frequencies $0 \leq f \leq 2W$, differences were only considered if the significance threshold was met over a contiguous frequency range from 0 to $\max(f, W) \leq 2W$.

To characterize the incidence of burst suppression or prolonged suppression in ASD vs. NT patients, we compared the proportion of ASD and NT patients displaying an episode of burst suppression or prolonged suppression using a Bayesian approach. We modeled this proportion as a beta distribution for each cohort wherein $\alpha = k + 1$ where $k = \#$ cases with confirmed events and $\beta = n + 1$ where $n = \#$ of cases in a given cohort. We estimated the posterior distribution for each cohort to represent the probability ($Pr$) that patients experience an episode of burst suppression or prolonged suppression. We then estimated the posterior density for the difference between $Pr_{ASD}$ and $Pr_{NT}$ ($\Delta Pr$) wherein $Pr_{ASD} > Pr_{NT}$ using a Markov Chain Monte Carlo approach. We chose a uniform prior distribution and used 10,000 Monte Carlo samples to compute the posterior density.
RESULTS

Aim 1. To characterize the propofol-induced frontal EEG in ASD pediatric patients.

Patient population

A total of 42 ASD patients aged 2 to 23 yr (10.88 ± 5.25 yr) were ultimately included in the EEG power spectra analysis. Patient baseline characteristics and anesthetic regimens are summarized in Table 1. Of note, 36 out of 42 (85.7%) of ASD patients were male, mirroring the known 4:1 male predominance of ASD. The comorbidity of clinically diagnosed epilepsy was 4 out of 42 (9.5%).

Table 1. Baseline characteristics and anesthetic regimens in ASD and NT pediatric cohorts. All values are means (SD). SD = standard deviation; SMD = standardized mean difference.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ASD (n=42)</th>
<th>NT (n=110)</th>
<th>SMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>10.88 (5.25)</td>
<td>13.29 (5.27)</td>
<td>0.460</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>36 (85.7)</td>
<td>61 (55.5)</td>
<td>0.704</td>
</tr>
<tr>
<td>Propofol infusion rate (mcg/kg/min) during epoch</td>
<td>239.88 (32.69)</td>
<td>256.11 (37.15)</td>
<td>0.464</td>
</tr>
<tr>
<td>Propofol bolus (mg/kg) prior to epoch</td>
<td>1.34 (1.13)</td>
<td>2.03 (1.52)</td>
<td>0.514</td>
</tr>
<tr>
<td>Midazolam (mg/kg) prior to epoch</td>
<td>0.01 (0.03)</td>
<td>0.00 (0.01)</td>
<td>0.430</td>
</tr>
<tr>
<td>Fentanyl (mcg/kg) prior to epoch</td>
<td>0.71 (0.62)</td>
<td>0.74 (0.58)</td>
<td>0.046</td>
</tr>
<tr>
<td>Comorbid clinically diagnosed epilepsy (%)</td>
<td>4 (9.5)</td>
<td>0 (0.0)</td>
<td>0.459</td>
</tr>
</tbody>
</table>

Differences in EEG power by age in ASD patients

Spectral analysis showed that total EEG power (0.1-40 Hz) under propofol anesthesia in ASD patients steadily declined from ages 2 to 23 yr. Based on these EEG power trends as well as visual inspection of the individual patient data and the age-varying spectrogram (Figure 10), we categorized the data as: 2-7 yr (Group 1, n=10), >7-12 yr (Group 2, n=17), >12-17 yr (Group 3, n=9), and >17-23 yr old (Group 4, n=6).
Figure 11 demonstrates the age-dependent frontal EEG in ASD patients in the aforementioned groups. Qualitatively, all groups displayed similar EEG spectral characteristics that were readily observed on EEG spectrogram, with large-amplitude slow-delta (0.1-4 Hz) and alpha (8-13 Hz) oscillations consistent with the use of propofol as the primary anesthetic agent. Compared to Groups 2 through 4, Group 1 displayed significantly increased power across a wide frequency range spanning slow to gamma frequencies (Table 2). Similarly, Group 2 had significantly increased power compared to Groups 3 and 4 in every frequency except for slow oscillations (0.1-1 Hz) (Table 2). Finally, Group 3 had significantly increased power compared to Group 4 across all frequencies (Table 2).
Figure 11. The age-dependent propofol-induced spectrogram and coherogram in ASD patients. (A) Median spectra and (B) median spectrograms of ASD patients by age demonstrate large power in the slow (0.1-1 Hz), delta (1-4 Hz), and alpha (8-13 Hz) frequencies present in all patients during propofol maintenance. The power of alpha oscillations begins to decline after age 12 yr. (C) Median coherence and (D) median coherograms of ASD patients by age demonstrate strong coherence in the alpha (8-13 Hz) frequency band present in all patients during propofol maintenance. There are no significant differences in coherence in age groups.

Table 2. Changes in power spectra by age group in ASD patients.

<table>
<thead>
<tr>
<th>Power Spectra (p&lt;0.0001, two group test spectra)</th>
<th>Group 1 (2-7yr) vs. Group 2 (&gt;7-12yr)</th>
<th>Group 1 (2-7yr) vs. Group 3 (&gt;12-17yr)</th>
<th>Group 1 (2-7yr) vs. Group 4 (&gt;17-23yr)</th>
<th>Group 2 (&gt;7-12yr) vs. Group 3 (&gt;12-17yr)</th>
<th>Group 2 (&gt;7-12yr) vs. Group 4 (&gt;17-23yr)</th>
<th>Group 3 (&gt;12-17yr) vs. Group 4 (&gt;17-23yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8-10.7 Hz; 17.6-25.9 Hz; 27.4-32.2 Hz; 33.7-42.0 Hz</td>
<td>Group 1&gt; 6.8-10.7 Hz; 17.6-25.9 Hz; 27.4-32.2 Hz; 33.7-42.0 Hz</td>
<td>Group 1&gt; 2.0-50 Hz;</td>
<td>Group 1&gt;0.1-50 Hz;</td>
<td>Group 2&gt;2.0-50 Hz;</td>
<td>Group 2&gt;1.5-50 Hz;</td>
<td>Group 3&gt;0.5-50 Hz;</td>
</tr>
</tbody>
</table>
Differences in EEG coherence by age in ASD patients

Overall, the age-dependent coherogram and coherence under propofol anesthesia did not vary significantly in ASD patients (Figure 11). Strong frontal alpha coherence was observed in all age groups across both cohorts. This is consistent with previous EEG studies of propofol in patients older than 1 yr. High alpha and low beta coherence was significantly higher in Group 1 than either Group 2 or Group 3, whereas slow-delta coherence was significantly increased in Group 4 relative to Group 1 (Table 3).

<table>
<thead>
<tr>
<th>Coherence (p&lt;0.0001, two group test coherence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (2-7yr) vs. Group 2 (&gt;7-12yr)</td>
</tr>
<tr>
<td>Group 1 (2-7yr) vs. Group 3 (&gt;12-17yr)</td>
</tr>
<tr>
<td>Group 1 (2-7yr) vs. Group 4 (&gt;17-23yr)</td>
</tr>
<tr>
<td>Group 2 (&gt;7-12yr) vs. Group 3 (&gt;12-17yr)</td>
</tr>
<tr>
<td>Group 2 (&gt;7-12yr) vs. Group 4 (&gt;17-23yr)</td>
</tr>
<tr>
<td>Group 3 (&gt;12-17yr) vs. Group 4 (&gt;17-23yr)</td>
</tr>
</tbody>
</table>

| Group 1 > 11.2-16.1 Hz                        |
| Group 1 > 12.2-16.1 Hz                        |
| Group 4 > 0.1-3.4 Hz                         |
| NA                                            |
| NA                                            |

Aim 2. To investigate the changes in the propofol-induced frontal EEG power in ASD vs. NT pediatric patients.

Patient population

A total of 110 NT patients aged 2 to 23 yr (13.30 ± 5.27 yr) were ultimately included in the EEG power spectra analysis to compare with ASD patients. Patient baseline characteristics and anesthetic regimens are summarized in Table 1.

There were notable differences in the characteristics of the ASD and NT cohorts. The mean age of ASD patients was less than that of NT patients (10.88 vs.13.30 yr, SMD = 0.460). ASD patients were also more likely to be male (85.7% vs. 55.5%, SMD = 0.704) and have comorbid epilepsy (4% vs. 0%, SMD = 0.459). Additionally, ASD patients received reduced propofol infusion rates (239.88 vs. 356.11 mcg/kg/min, SMD
= 0.464) and propofol boluses (1.34 vs. 2.03 mg/kg, SMD = 0.514), but increased midazolam premedication (0.01 vs. 0.00 mg/kg, SMD = 0.430). There were no substantial differences in fentanyl administration in ASD vs. NT patients (0.71 vs. 0.74 mcg/kg, SMD = 0.046).

**Modeling age-dependent EEG power in ASD vs. NT patients**

To assess the age-dependent changes in power in the propofol-induced EEG in ASD vs. NT patients, we used a Bayesian approach to investigate the relationship of EEG power and age in alpha (8-13 Hz) and slow (0.1-1 Hz) oscillations, while accounting for baseline characteristics. These two oscillatory bands were chosen as they represent the primary EEG signals induced by propofol administration. The data were assessed using Markov Chain Monte Carlo techniques. Two separate models were utilized to examine alpha and slow waves. The primary linear regression models assumed no prior probabilities and analyzed differences in power between ASD and NT groups while adjusting for gender, propofol infusion rate (mcg/kg/min), propofol bolus (mg/kg), midazolam (mg/kg), fentanyl (mg/kg), and comorbidity of epilepsy to validate and visualize our findings.

To assess the robustness of the model, we performed 100 simulations of a modal patient varying by age, group (ASD or NT), and band (alpha or slow) and compared the results with a line of best fit representation through the raw data (Supplementary Figure 3).

**The trajectory of alpha (8-13 Hz) power by age differs in ASD vs. NT patients**

*Figure 12* shows the trajectory of alpha power by age in ASD vs. NT pediatric patients after accounting for differences in baseline characteristics between the cohorts. ASD patients at mean age experienced a non-significant 0.47 dB reduction in alpha power compared to NT patients (80% credibility interval -1.82 to 0.82) (*Table 4*).
Figure 12. Alpha (8-13 Hz) power in the propofol-induced frontal EEG by age and ASD status.
### Table 4. Posterior probabilities from primary model of alpha (8-13 Hz) power (dB) by using mean age. HPD = highest posterior density

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alpha (8-13 Hz) Power (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>Group= ASD</td>
<td>-0.469</td>
</tr>
<tr>
<td>Age</td>
<td>-0.077</td>
</tr>
<tr>
<td>Age2</td>
<td>0.000</td>
</tr>
<tr>
<td>Age3</td>
<td>0.000</td>
</tr>
<tr>
<td>Gender=1</td>
<td>0.169</td>
</tr>
<tr>
<td>PropofolInfusion</td>
<td>-0.013</td>
</tr>
<tr>
<td>PropofolBolus</td>
<td>-0.161</td>
</tr>
<tr>
<td>Midazolam</td>
<td>-5.937</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.761</td>
</tr>
<tr>
<td>Epilepsy_comorbid=1</td>
<td>0.885</td>
</tr>
<tr>
<td>Group*Age</td>
<td>0.004</td>
</tr>
<tr>
<td>Group*Age2</td>
<td>0.000</td>
</tr>
<tr>
<td>Group*Age3</td>
<td>0.000</td>
</tr>
<tr>
<td>sigma2</td>
<td>11.293</td>
</tr>
</tbody>
</table>

However, there were notable differences in alpha power in ASD vs. NT patients at specific ages. At age 65mo (5.42yr), the mean alpha power was increased in NT (18.86 ± 0.68 dB) vs. ASD patients (17.65 ± 0.89 dB) with 56.5% certainty (Figure 13A). At age 270mo (22.5yr), the mean alpha power was increased in NT (11.89 ± 1.77 dB) vs. ASD patients (6.37 ± 2.50 dB) with 80.2% certainty (Figure 13B).
Figure 13. Posterior distributions of alpha (8-13 Hz) EEG power (dB) at (A) 65 months and (B) 270 months in ASD vs. NT cohorts.

The trajectory of slow (0.1-1 Hz) power by age differs in ASD vs. NT patients

Figure 14 shows the trajectory of slow power by age in ASD vs. NT pediatric patients after accounting for baseline characteristics between the cohorts, ASD patients at mean age experienced a significant 1.93 dB reduction in slow power compared to NT patients (80% credibility interval -3.38 to -0.54) (Table 5).
Figure 14. Slow (0.1-1 Hz) power in the propofol-induced frontal EEG by age and ASD status.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>50%</th>
<th>80% HPD Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group= ASD</td>
<td>-1.926</td>
<td>(-3.382, -0.538)</td>
</tr>
<tr>
<td>Age</td>
<td>-0.024</td>
<td>(-0.041, -0.008)</td>
</tr>
<tr>
<td>Age2</td>
<td>0.000</td>
<td>(-0.000, 0.000)</td>
</tr>
<tr>
<td>Age3</td>
<td>0.000</td>
<td>(-0.000, 0.000)</td>
</tr>
<tr>
<td>Gender=1</td>
<td>-0.428</td>
<td>(-1.255, 0.437)</td>
</tr>
<tr>
<td>PropofolInfusion</td>
<td>0.017</td>
<td>(0.006, 0.029)</td>
</tr>
<tr>
<td>PropofolBolus</td>
<td>0.699</td>
<td>(0.386, 0.960)</td>
</tr>
<tr>
<td>Midazolam</td>
<td>36.503</td>
<td>(14.701, 57.802)</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>-1.366</td>
<td>(-2.036, -0.684)</td>
</tr>
<tr>
<td>Epilepsy_comorbid=1</td>
<td>3.246</td>
<td>(0.817, 5.729)</td>
</tr>
<tr>
<td>Group*Age</td>
<td>0.009</td>
<td>(-0.024, 0.041)</td>
</tr>
<tr>
<td>Group*Age2</td>
<td>0.000</td>
<td>(-0.000, 0.000)</td>
</tr>
<tr>
<td>Group*Age3</td>
<td>0.000</td>
<td>(-0.000, 0.000)</td>
</tr>
<tr>
<td>sigma2</td>
<td>13.093</td>
<td>(11.1202, 15.328)</td>
</tr>
</tbody>
</table>

There were also notable differences in slow power in ASD vs. NT patients at specific ages. At age 28mo (2.33yr), the mean slow power was increased in ASD (17.05 ± 2.20 dB) vs. NT patients (14.20 ± 1.83 dB) with 52.5% certainty (Figure 15A). At age 130mo (10.8yr), the mean slow power was increased in NT (18.99 ± 0.70 dB) vs. ASD patients (16.93 ± 0.87 dB) with 80.8% certainty (Figure 15B). At age 270mo (22.5yr), the mean slow power was increased in NT (13.95 ± 1.91 dB) vs. ASD patients (11.56 ± 2.69 dB) with 41.3% certainty (Figure 15C).
Aim 3. To characterize the incidence of burst suppression and prolonged suppression in the propofol-induced frontal EEG in ASD vs. NT pediatric patients

Patient population

For the burst suppression analysis, the inclusion criteria were expanded to include patients between ages 23 to 30 yr, as well as patients who had been in burst suppression throughout the case and thus were not able to undergo power spectra analysis. This expanded the total cohorts studied to 56 ASD patients aged 2 to 30 yr and 123 NT patients aged 2 to 30 yr.

Increased incidence of suppression events in ASD vs. NT patients

ASD patients experienced an episode of burst suppression or prolonged suppression nearly twice as often as NT patients (23% vs. 12.2%, p<0.05). The
probability that ASD patients had greater incidence of burst suppression or prolonged suppression was 0.9730 (Figure 16).

![Figure 16](image)

**Figure 16. The probability of suppression events is significantly increased in ASD patients.** (A) Posterior distributions of the probability of burst suppression in ASD and NT cohorts. Data was fit to the beta distribution with parameters (15,43) and (16,109) for ASD and NT cohorts, respectively. (B) Histogram of the difference between the probability of burst suppression in ASD and NT cohorts, where $P_{ASD} > P_{NT}$. A Monte Carlo simulation was performed by drawing 10,000 random samples from each posterior distribution. The probability that $P_{ASD} > P_{NT}$ was 0.9834.

**Clinical characteristics in ASD vs. NT patients experiencing suppression events**

We next investigated the clinical characteristics of all cases with confirmed burst suppression or prolonged suppression to explore the potential causes of this finding (Table 6). The mean age of ASD patients was moderately higher than NT patients (17.48 vs. 14.67 yr, SMD = 0.459), as was the rate of comorbid epilepsy (21.4% vs. 0%, SMD = 0.739). Interestingly, there was a similar male predominance in both ASD and NT cohorts (71.4% vs. 73.3%, SMD = 0.043). ASD patients received reduced total propofol infusion dose (2.59 vs. 3.26 mg/kg, SMD = 0.281) and propofol bolus dose (1.34 vs. 2.01 mg/kg, SMD = 0.424) prior to the onset of burst suppression compared to NT patients. ASD patients received increased midazolam premedication dose (0.01
vs. 0.00 mg/kg, SMD = 0.561) prior to the onset of burst suppression compared to NT patients. There were no substantial differences in fentanyl dose prior to onset of burst suppression in ASD vs. NT patients (0.57 vs. 0.70 mcg/kg, SMD = 0.141).

Table 6. Baseline characteristics and anesthetic regimen in ASD and NT patients who experienced burst suppression or prolonged suppression. All values are mean (SD). SD = standard deviation; SMD = standardized mean difference

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ASD (n=14)</th>
<th>NT (n=15)</th>
<th>SMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>209.71 (66.50)</td>
<td>176.93 (76.16)</td>
<td>0.459</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>10 (71.4)</td>
<td>11 (73.3)</td>
<td>0.043</td>
</tr>
<tr>
<td>Propofol infusion (mg/kg) prior to suppression</td>
<td>2.59 (2.23)</td>
<td>3.26 (2.58)</td>
<td>0.281</td>
</tr>
<tr>
<td>Propofol bolus (mg/kg) prior to suppression</td>
<td>1.34 (1.77)</td>
<td>2.01 (1.38)</td>
<td>0.424</td>
</tr>
<tr>
<td>Midazolam (mg/kg) prior to suppression</td>
<td>0.01 (0.03)</td>
<td>0.00 (0.00)</td>
<td>0.561</td>
</tr>
<tr>
<td>Fentanyl (mcg/kg) prior to suppression</td>
<td>0.57 (1.21)</td>
<td>0.70 (0.57)</td>
<td>0.141</td>
</tr>
<tr>
<td>Comorbid clinically diagnosed epilepsy (%)</td>
<td>3 (21.4)</td>
<td>0 (0.00)</td>
<td>0.739</td>
</tr>
</tbody>
</table>
DISCUSSION

The prevalence of ASD has risen dramatically over the past decade, spurring a rapid call to action to determine the underlying cause of the disorder as well as the optimal ways to care for children with ASD. In this study, we sought to elucidate changes in the development and function of GABAergic inhibitory circuits that are thought to go awry in ASD by studying the EEG under propofol anesthesia. While previous studies investigating the pathophysiology of ASD have employed genetic screening, awake and sleep EEG, and numerous imaging modalities, the anesthesia-induced EEG offers a unique, non-invasive way of measuring the brain’s response to a specific neuro-pharmacological probe that is known to act at GABAergic sites that are thought to be central to the etiology of ASD. To our knowledge, this study represents the first use of the anesthesia-induced EEG to explore the underlying pathophysiology of ASD. In addition, our results are among the first to shed light on how anesthetic management could be adjusted to account for a patient’s ASD status. Currently, other than receiving premedication at a higher rate, ASD patients are often managed identically to NT patients in terms of medication choice and dosing for GA and sedation.

Here, we demonstrate significant changes in the age-dependent propofol-induced frontal EEG in ASD vs. NT pediatric patients. After adjusting for clinical characteristics, the trajectory of both slow (0.1-1 Hz) and alpha (8-13 Hz) EEG power by age was significantly different in the ASD vs. NT patients. In particular, both alpha and slow power were significantly reduced in older ASD vs. NT patients. Additionally, the incidence of burst suppression was significantly increased in ASD vs. NT patients (23.6% vs. 12.2%, p<0.05). This occurred despite significantly reduced total doses of propofol given via infusion and boluses prior to suppressive events in ASD vs. NT patients. We explore the implications of these findings in the following sections, as well as the potential for future work.
Changes in the Age-Dependent Propofol-Induced EEG in ASD

The EEG measures scalp electrical potentials generated by cortical post-synaptic currents. In adults, propofol induces stereotyped EEG oscillations that reflect the functional disruption of cortical and thalamocortical circuits. Propofol-induced slow oscillations reflect alternating “up” and “down” states in cortical neurons whose firing is silenced by periods of hyperpolarization. Propofol-induced frontal alpha oscillations are thought to arise from amplification of inhibitory GABAergic signaling within thalamocortical circuits. In NT children, the propofol-induced EEG shows a striking age dependence. The frontal alpha oscillations are absent in infants, but develop within the first year of life. The appearance of coherent frontal alpha oscillations on propofol-induced EEG in children less than 1 yr mirrors this time course, suggesting that thalamocortical connectivity is necessary for spatially coherent anesthesia-induced alpha oscillations. Both propofol-induced frontal alpha and slow oscillations increase in power through the first 8 to 10 years of life, decreasing subsequently through adolescence and adulthood. These age-related changes are thought to reflect the trajectory of postnatal neurodevelopment, particularly within frontal cortical and thalamocortical circuits.

Overall, the propofol-induced frontal EEG in ASD patients closely resembled that of NT patients undergoing propofol anesthesia. Prominent slow-delta and frontally coherent alpha oscillations were present in all age groups in the ASD cohort, similar to NT patients. This preserved EEG appearance suggests that the brain networks responsible for generating the characteristic “signature” of propofol-induced oscillations are intact in ASD. Accordingly, there is no clinical evidence of ASD patients experiencing an impairment in propofol-induced unconsciousness either anecdotally or in the literature. We also observed a progressive reduction in total EEG power (0.1-40Hz) by age, with no or little differences in coherence by age. This, too, is grossly similar in to our prior results in NT patients.

However, in ASD patients, the age dependence in these features was different from NT patients. Slow oscillation power was initially higher in ASD vs. NT patients (17.05 vs. 14.20 dB at 2.33yr), but progressively declined with age (11.56 vs. 13.95 dB
at 22.5 yr). Frontal alpha power was initially lower in ASD vs. NT patients (17.65 vs. 18.86 dB at 5.42yr) and continued to decline with age (6.37 vs. 11.89 dB at 22.5yr).

The changes in the age-dependent propofol-induced frontal EEG observed in ASD patients could reflect underlying neuropathology associated with ASD. Post-mortem studies have shown excessive cortical synaptic density in very young children with ASD.99-102,106 This higher cortical synaptic density could correspond to higher postsynaptic currents and increased slow oscillation EEG power. Accelerated frontal cortical thinning has also been reported in adolescent ASD patients,106,107 and the progressive reduction in slow EEG power in ASD could correspond with this cortical thinning. Alternatively, interruption of synaptic refinement may lead to the formation of poorly functioning circuits, which may impair generation of anesthesia-induced slow oscillations in later childhood and adolescence relative to NT patients. Disruption of thalamocortical functional and anatomical connectivity has also been shown in older ASD patients.118 The progressive decline in alpha EEG power in ASD could correspond with this disrupted connectivity.

Increased Probability of Burst Suppression and Prolonged Suppression in ASD

Burst suppression can be observed in hypothermia, hypoxia, coma, congenital forms of epilepsy such as Ohtahara syndrome and early infantile encephalopathy.119-124 Most general anesthetics that act primarily by enhancing GABAergic transmission can induce burst suppression, including the halogenated ethers, propofol, and barbiturates.125-130 The mechanisms for burst suppression are poorly understood; one hypothesis suggests that it may be caused and facilitated by the activity of adenosine triphosphate (ATP)-gated potassium channels, which open in response to the low intracellular levels of ATP during bursts, resulting in hyperpolarization and a cessation of action potentials.27 Another hypothesis suggests that the suppression periods could be provoked by increased cortical excitability that depletes extracellular calcium.27,131 As the dose of the anesthetic is increased, the length of the suppression periods between the bursts increases. The dose can be increased to the point at which the EEG is isoelectric.
The typical developmental shift of GABA from an excitatory to inhibitory neurotransmitter depends on sequential maturation of the bumetanide-sensitive sodium-(potassium)-chloride co-transporter 1 (NKCC1) to potassium-chloride co-transporter 2 (KCC2).\textsuperscript{78,79} ASD patients have been shown to have an increased NKCC1:KCC2 ratio, which tends to preserve excitatory GABAergic signaling but also leads to increased intracellular potassium and chloride in affected neurons.\textsuperscript{80-83} This would increase the likelihood of burst suppression in the theoretical model described above. Administration of bumetanide (an NKCC1 inhibitor) has also been shown to reduce ASD symptoms in both human patients and animal models of ASD.\textsuperscript{80-83}

Taken together, reduced alpha oscillation power and increased probability of burst suppression in ASD patients during propofol anesthesia appears to be similar to the pattern observed in elderly NT adults.\textsuperscript{52} Our results may therefore signify a form of accelerated neuronal aging during the late adolescent years in ASD patients.

Intraoperative burst suppression has been shown to be an independent risk factor for postoperative delirium (POD), with longer times spent in burst suppression corresponding to a higher risk.\textsuperscript{132,133} However, the adverse effects of burst suppression during GA have only been investigated in elderly patients. This study suggests that it may be appropriate to investigate the incidence of and consequences of burst suppression in other potentially vulnerable patient populations, including neurodevelopmental disease such as ASD.

**Limitations**

While the anesthesia-induced EEG readout provided a noninvasive and versatile way to provide insights into the differences between ASD and NT patients, the need to be mindful of clinical standards of care also created natural limitations to this study. Below we describe several limitations, along with an explanation of our strategy to minimize the limitations.

First, a limitation of this study is that medications were administered by clinical judgment of the anesthesiologist, and thus dosing was neither prospective nor controlled. As a result, it is possible that differences in the age-dependent propofol-induced EEG observed in this study could reflect differences in anesthetic
management, rather than differences in underlying neurobiology. However, after accounting for differences in medication administration in our modeling, we still found significant changes in EEG power by age in ASD vs. NT patients. Furthermore, in all cases, all anesthesia and adjuncts were administered to maintain a state of GA required for the procedural stimuli. This suggests that all patients were in a similar neurophysiologic state at the chosen epoch for analysis.

Our analysis revealed that there were moderate effect size differences of propofol infusion rate (mcg/kg/min), propofol bolus dose (mg/kg), and midazolam (mg/kg) dose between the ASD and NT cohorts in both the EEG power and suppression analyses. ASD patients received reduced amounts of propofol (both infusion and bolus) and higher amounts of midazolam relative to NT patients. Based on our understanding of anesthesia-induced EEG changes in adult patients, reduced propofol dosing could result in a state of lessened unconsciousness that would tend to diminish alpha and slow oscillations with elevation of beta/gamma (13-50 Hz) oscillations.9 However, this is not in agreement with the result that ASD patients also had an increased incidence of burst suppression, a state of profound unconsciousness beyond that required for GA. Furthermore, the age-varying spectrogram (Figure 10) did not reveal a concomitant rise in beta-gamma oscillations in the ASD population. While this was not explicitly quantified in our model, it is unlikely that significant differences in the beta/gamma oscillations are present as we note that reductions of both alpha and slow oscillations are visible in Figure 10.

ASD patients were also noted to have increased midazolam dosing. Administration of benzodiazepines tends to induce beta (13-25 Hz) oscillations as well as reduce slow (0.1-1 Hz) and alpha (8-13 Hz) oscillations in awake subjects.134 Benzodiazepines, like other GABAergic agents, can also increase the likelihood of burst suppression but do not precipitate it as a single agent.135 However, only a small subset of ASD patients in our study actually received midazolam premedication. The magnitude of the effect size is due to the fact that almost no NT patients received midazolam premedication. Furthermore, the average dose was clinically insignificant at 0.01mg/kg, which corresponds to about 0.31mg in an average 10yr male patient
(31kg). For reference, the average dose of IV midazolam for preoperative anxiolysis is 0.05-0.10mg/kg for all pediatric patients. Thus, the dosing recorded would not be expected to produce EEG changes of the magnitude observed in our study. Ultimately, this reduces the likelihood that differences in medication dosing or other clinical characteristics caused the observed changes in EEG power in ASD patients.

Another limitation is that ASD is an incredibly heterogeneous disease, and the patients represented in this study experienced a broad range of symptom severity as well as comorbidities. Additionally, many patients with ASD take psychoactive medications such as antiepileptics, antidepressants, and antipsychotics chronically, which could plausibly affect the EEG dynamics observed. However, we believe that our ASD cohort is representative of the general ASD population presenting for anesthesia. To impose excessively narrow criteria would detract from an understanding of the general pathophysiology of ASD and differences in clinical management for these patients. Indeed, it is remarkable that we were able to find significant differences in the age-dependent propofol-induced EEG despite the inherent variability of ASD and clinical circumstances. This indicates that the neurophysiological effects must be very robust.

In the suppression analysis, the mean age of ASD patients was moderately higher than NT patients (17.48 vs. 14.67 yr, SMD = 0.459), as was the rate of comorbid epilepsy (21.4% vs. 0%, SMD = 0.739). Both of these characteristics could contribute to an increased probability of experiencing suppressive events. However, it is again unlikely that the magnitude of difference in incidence of suppression events between ASD and NT patients could be attributed to these qualities alone. In our previous study of elderly patients compared to young patients, a 2-fold difference in burst suppression incidence only occurred over two decades (20 yr to 40 yr).

No awake baseline EEG was recorded in any case for comparison to the anesthesia-induced EEG. However, this technically difficult in NT pediatric patients without the use of sedation or GA, and is nearly impossible in ASD pediatric patients with hypersensitivities to sensory stimuli.
Finally, we may not have accounted for all variables that could affect EEG power or burst suppression occurrence in our modeling, and thus potential confounding factors may still exist. Additionally, we had a limited sample size and as our modeling was sample-dependent, this may also obscure the results.

**Implications for Anesthetic Management in ASD**

These findings have important implications for clinical monitoring and management of GA and sedation in patients with ASD. Although there were notable differences as discussed, ASD patients had qualitatively similar propofol-induced oscillations to NT patients suggesting a preserved neurophysiological mechanism for generating these oscillations. While defined behavioral endpoints such as loss of response and loss of consciousness were not explored in this study, this implies that we can harness the anesthesia-induced EEG to monitor unconsciousness in children with ASD undergoing GA, similar to NT patients. This enables clinicians to tailor their anesthetic regimen for ASD patients using real-time depth of anesthesia monitoring, which prevents adverse outcomes such as under- or oversedation and burst suppression. In particular, preventing excessive anesthetic may prevent postoperative delirium, postoperative cognitive dysfunction, and further brain damage in vulnerable cohorts. This study also demonstrates how clinical management should be more nuanced to account for how brain health may affect response to a given anesthetic. Given our results as well as previous evidence of the structural CNS abnormalities over time in ASD, we propose that ASD patients need reduced doses of GABAergic agents compared to NT patients. This is particularly important as the majority of physicians at our hospital believe that ASD patients require higher doses of anesthetic, when in fact they may only require premedication more frequently, and thus are at high risk for oversedation and burst suppression.

**The Anesthesia-Induced EEG as a Novel Tool to Assess Brain Health**

Additionally, our study is serves as a proof-of-concept that the anesthesia-induced EEG can be employed as a tool to assess the health of underlying brain
circuits. Our previous work explored how elderly NT patients generate significantly different EEG oscillations during propofol- and sevoflurane-induced unconsciousness compared to young patients, and are more likely to enter burst suppression. This was thought to represent progressive cortical thinning and altered metabolism in the aging brain. Here, we demonstrate that the propofol-induced EEG in ASD patients displays similar differences, suggestive of the underlying deficits in GABAergic circuits acted upon by propofol. This study highlights that the anesthesia-induced EEG can be harnessed to assess other disorders of neurodevelopment and neurodegeneration such as schizophrenia, Alzheimer’s disease, and anesthesia-induced neurotoxicity in children. As with ASD, this approach can augment our understanding of the pathophysiology of each disorder and changes that may be necessary in anesthetic management for these patients. Anesthetics act as a stimulus, engaging a dynamic system in a way that can reveal problems with gross systems-level response in patients with brain disease.
CONCLUSION

Here we present evidence that ASD patients respond differently to propofol, a GABAergic agent, compared to NT patients. We first explored the evolution of the propofol-induced frontal EEG with age in the ASD cohort, finding qualitatively similar propofol-induced oscillations suggesting a preserved mechanism for generating these oscillations. We next characterized age-dependent changes in slow (0.1-1 Hz) and alpha (8-13 Hz) EEG power in ASD vs. NT patients. After adjusting for clinical characteristics, the trajectory of both slow and alpha EEG power by age was significantly different in the ASD vs. NT patients. In particular, both alpha and slow power were reduced in older ASD vs. NT patients. Additionally, the incidence of burst suppression was significantly increased in ASD vs. NT patients (23.6% vs. 12.2%, p<0.05). This occurred despite significantly reduced total doses of propofol given via infusion and boluses prior to suppressive events in ASD vs. NT patients.

In conclusion, our results demonstrate clear changes in age-dependent propofol-induced EEG oscillations that may reflect increased cortical thinning and neurodegeneration with age in cortical and thalamocortical circuitry in ASD. Although future studies will be needed to provide greater insight into the basis of these changes, this study provides compelling preliminary evidence that changes in brain development in ASD can be non-invasively assessed using the EEG. As a similar pattern of decreased alpha power and increased sensitivity to burst suppression develops progressively with age in NT adults, our results may also signify a form of accelerated neuronal aging during the late teenage years in ASD patients. Taken together, we demonstrated that characterizing changes in brain states induced by anesthetic drugs can enable insights into the underlying differences in neural circuitry of ASD, as well as yield safer practices for managing anesthetic care of ASD patients.
SUGGESTIONS FOR FUTURE WORK

Future directions include the use of prospective, controlled-dose experiments with targeted behavioral assessments such as timing of loss of consciousness, delayed emergence, presence of post-operative delirium, or quantitative assessment of the severity of autism symptomatology. The optimal confirmation of equivalent propofol doses would be blood- and effect-site (i.e., CNS) concentrations of propofol in each subject, but this is likely not feasible in most operating room settings.

In order to further decrease the effects of neurodevelopmental variability in ASD, it may be prudent to impose narrower selection criteria on our study population based on gender, genetic testing, presence of intellectual disability, presence of clinical epilepsy, acquisition of verbal language, and other parameters. In our ASD study population, we included patients of both genders and did not exclude for clinical epilepsy, intellectual disability, or non-verbal status, which could all theoretically account for differences in the ASD vs. NT cohorts beyond ASD status. However, there is a risk that the selected subpopulation may not reflect the disorder as a whole. Alternatively, we want to recruit a larger number of patients to minimize the effect that such variability would have on the overall results. This would likely require an extensive multicenter trial to attain an adequate number of patients.

It would be ideal to have EEG data from both the awake and anesthetized state. The awake EEG generally requires that the subject can open and close their eyes periodically over approximately 10 minutes. While the awake EEG can be technically difficult in pediatric patients, particularly those with extreme sensitivities to sensory stimuli, other laboratories have accomplished this with gradual introduction of the device through multiple appointments leading up to an official recording. Additionally, the use of full-head EEG monitors would improve our understanding of the underlying circuitry compared to the use of frontal EEG monitors such as the SEDLine device. This could be accomplished through use of devices such as the Enobio 32 wireless EEG acquisition system with pediatric caps (Neuroelectrics, Barcelona, Spain) or the Waveguard® wired EEG pediatric cap with the ASA-Lab EEG acquisition system, which have been used for previous studies in our institution.
To further delineate the basis of the changes in the propofol-induced EEG, it would be interesting to develop animal models of ASD and high-density EEG or MRI studies in ASD patients to better compare the location and activity of circuits leading to the development of slow and alpha oscillations during propofol-induced unconsciousness in ASD vs. NT patients.

Future studies in our laboratory will likely invoke the anesthesia-induced EEG as a tool to explore other disorders of neurodevelopment and neurodegeneration such as schizophrenia, Alzheimer’s disease, and anesthesia-induced neurotoxicity in children. As with ASD, this approach can augment our understanding of the pathophysiology of each disorder.

Clinically, this study provides an example of how the EEG can be used to provide real-time feedback on a patient’s brain state during general anesthesia, allowing anesthesiologists to provide personalized anesthesia care for their patients. In addition to using the anesthesia-induced EEG as a probe to explore the pathophysiology of other neurodevelopmental and neurodegenerative diseases, anesthesiologists should embrace the anesthesia-induced EEG as a method of monitoring brain states in patients at potential risk for suboptimal anesthetic.
SUMMARY

General anesthetic drugs induce characteristic oscillations within brain circuits that can be readily assessed using the electroencephalogram (EEG), allowing a controlled and noninvasive way to study the circuitry of the human brain. Previous studies from our laboratory show significant age-related changes in the propofol-induced frontal EEG thought to reflect the development of underlying circuits mediated by GABAergic interneurons. Autism spectrum disorder (ASD) is thought to arise from deficits in GABAergic signaling leading to abnormal neurodevelopment and neuromodulation, but no one has characterized how these underlying changes may influence response to a GABAergic agent. This study determined that the trajectory of age-dependent alpha (8-13 Hz) and slow (0.1-1 Hz) power in the propofol-induced frontal EEG showed notable differences ASD vs. NT patients. It also found that the incidence of burst suppression or prolonged suppression was doubled in ASD vs. NT patients despite lower propofol doses. These results suggest that ASD patients respond differently to the GABAergic drug propofol and may have lowered anesthesia requirements for GABAergic agents compared to NT patients. These differences and their age-dependence may reflect underlying differences in GABAergic circuit function and development in ASD. As a similar pattern of decreased alpha power and increased sensitivity to burst suppression develops progressively with age in NT adults, our results may signify a form of accelerated neuronal aging during the late teenage years in ASD patients. Taken together, our results suggest that measuring the frontal EEG in ASD patients may enable insights into the underlying differences in neural circuitry of ASD, as well as yield safer practices for managing anesthetic care of ASD patients.
Supplementary Figure 1. Neurophysiological basis of EEG. A. Normal communication in the brain through neuronal spiking activity induces oscillatory extracellular electrical currents and potentials that are one of the ways information exchange is modulated and controlled in the central nervous system. B. The geometry of the neurons in the cortex favors the production of large extracellular currents and potentials. C. The electroencephalogram recorded on the scalp is a continuous measure of the electrical potentials produced in the cortex. D. Because the cortex (orange region) is highly interconnected with subcortical regions, such as the thalamus (yellow region), and the major arousal centers in the basal forebrain, hypothalamus, midbrain and pons, profound changes in neural activity in these areas can result in major changes in the scalp electroencephalogram.\(^9,137\)
Supplementary Figure 2. Schematic of spectral analysis. (A) The raw EEG signal is measured in $\mu$V over time. This is called the time series signal. (B) The raw signal can be separated into low- and high-frequency components. (C) The amplitude of each frequency can be calculated at a specific point in time. This is called the spectrum. (D) Repetition of the frequency decomposition over time leads to the formation of a 3-dimensional plot that represents the collection of spectra over time ($x$-axis). This is called the spectrogram. (E) The power dimension of the 3-D spectrogram can be represented as a colorized scale, which indicates the amplitude of a specific frequency ($y$ axis) in the signal at a given time ($x$ axis). This creates the 2-D spectrogram. (Courtesy of Patrick L. Purdon)
Supplemental Figure 3. Demonstration of fit in age-dependent EEG power model. Raw data (top panel) compared to modal patient simulation (bottom panel) in the Bayesian model of alpha (8-13 Hz) and slow (0.1-1 Hz) EEG power by age and ASD status.
CITATIONS


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