Multiple Anesthetic Exposure in Infant Monkeys Alters Emotional Reactivity to an Acute Stressor

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ABSTRACT

Background: Retrospective studies in humans have shown a higher prevalence of learning disabilities in children that received multiple exposures to general anesthesia before the age of 4 yr. Animal studies, primarily in rodents, have found that postnatal anesthetic exposure causes neurotoxicity and neurocognitive deficits in adulthood. The authors addressed the question of whether repeated postnatal anesthetic exposure was sufficient to cause long-term behavioral changes in a highly translationally relevant rhesus monkey model, allowing study of these variables against a background of protracted nervous system and behavioral development.

Methods: Rhesus monkeys of both sexes underwent either three 4-h exposures to sevoflurane anesthesia (anesthesia group n = 10) or brief maternal separations (control group n = 10) on postnatal day 6 to 10 that were repeated 14 and 28 days later. Monkeys remained with their mothers in large social groups at all times except for overnight observation after each anesthetic/control procedure. At 6 months of age, each monkey was tested on the human intruder paradigm, a common test for emotional reactivity in nonhuman primates.

Results: The frequency of anxiety-related behaviors was significantly higher in monkeys that were exposed to anesthesia as neonates as compared with controls: anesthesia 11.04 ± 1.68, controls 4.79 ± 0.77, mean ± SEM across all stimulus conditions.

Conclusion: Increased emotional behavior in monkeys after anesthesia exposure in infancy may reflect long-term adverse effects of anesthesia. **(ANESTHESIOLOGY 2015; 123:1084-92)**

RETROSPECTIVE birth-cohort studies have reported a significant increase in learning disability, incidence of attention deficit/hyperactivity disorder, and reduced performance on cognitive tests and academic achievement in children who received more than one general anesthetic before the age of 4 yr.[1–3] Other studies have reported impairments in specific cognitive domains after a single exposure to anesthesia in childhood.[4–6] Rodent and nonhuman primate studies have demonstrated that general anesthetics (including ketamine, nitrous oxide, propofol, isoflurane, and sevoflurane) cause persistent brain damage and learning deficits when administered during early postnatal development.[7–19] In fact, the highest rates of cell death after exposure to general anesthesia are found in the prefrontal cortex, amygdala, and hippocampus,[7,12,13,16,20,21] which are brain areas important for normal social, emotional, and cognitive functioning. These data from animal studies suggest that changes in cognitive and emotional behavior after anesthesia exposure in children could be due directly to the impact of anesthetics on the developing brain.

Nevertheless, uncertainty remains about the extent to which anesthesia specifically may be a risk factor for neurocognitive impairment in humans when compared with other factors such as comorbidity. Moreover, the applicability of studies in rodents to humans has been questioned on

What We Already Know about This Topic

- Preclinical studies in rodents have demonstrated that multiple anesthetic exposures during the brain growth spurt leads to neuronal degeneration and cognitive deficits in adulthood.
- A significant concern is whether findings in rodent studies are applicable to humans given the substantial differences in brain developmental stages between species.
- To develop a model that has greater translational potential, the authors used a multiple sevoflurane exposure paradigm in rhesus monkeys at time of brain development in monkeys that is equivalent to that of a 6-month-old human infant.

What This Article Tells Us That Is New

- Rhesus monkey infants subjected to three 4-h exposures to sevoflurane manifested increased anxious behavior when confronted with an intruder. The increased anxiety was not attributable to changes in physiologic function during anesthesia.
- Vocalizations, fear, and irritable behavior, in an age-specific manner, were similar between exposed and nonexposed monkeys.
- Within a spectrum of behavioral changes induced by neonatal anesthetic exposure, attention should be focused on altered emotional behavior.

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a number of grounds, including physiological status during anesthesia and the correspondence of developmental stages between the species.[22,23] By contrast, the stage of neurodevelopment of rhesus monkeys at birth is more similar to that of human infants compared with neonatal rodents.[16] For instance, in a comparison of rate of brain growth, humans peak around the time of birth, whereas rhesus monkeys peak prenatally, and rhesus monkeys are at a phase at birth comparable with that of a 6-month-old human.[16,24] Thus, at least with respect to brain growth spurt, our infant monkeys that are exposed to anesthesia in the first 6 weeks of life correspond to humans in the second half of their first year of life. Thus, anesthetic exposure during the first few weeks of life of a monkey may better model the human condition, at least that of human infants older than 6 months. Furthermore, it is possible to maintain normal physiology (normocapnia, normoxia, and normotension) to a much greater extent in infant monkeys than in rodents, abrogating concerns that cell death and impaired neurocognitive development after anesthetic exposure in rodents may be attributable to hypercapnia[25] or other physiological factors. Finally, the development of monkeys is protracted in time (compared with rodents, who are sexually mature around 6 to 7 weeks of age), thus it is possible to observe whether any effects of anesthesia emerge (or disappear) at different points during early development.

The current study compares the impact of multiple exposures to sevoflurane on cognitive and emotional behavior in infant rhesus monkeys with that of normally developing controls. Sevoflurane was chosen as the agent for study because it is a common pediatric anesthetic. It is also consistently associated with cell death when given early in development in rodents[10,12,26] and has been associated with abnormal socioemotional behavior in mice.[19] We exposed monkeys to sevoflurane three times, beginning approximately at postnatal day 7 and then again 2 and 4 weeks later because repeated anesthesia exacerbates the risk for cognitive impairment.[1,2,18] We chose a 4-h anesthetic to model the length of anesthesia that would be required for a significant surgical procedure in humans (*e.g.*, the mean duration of anesthesia was 125 min in the study by Wilder) and to increase the likelihood that significant cell death and therefore long-term neurodevelopmental effects will occur. Five hours of isoflurane is sufficient to induce extensive apoptosis in neonatal macaque neocortex,[16] as is 9 h (but not 3 h) of continuous ketamine infusion.[15] This balances a duration of anesthesia that is long enough to induce neuronal cell death but still within the window of anesthetic durations that would be given clinically. We chose repeated anesthetic exposure because the human data indicate that repeated anesthesia results in learning disabilities even though single anesthetics do not.[1] Thus, we have chosen parameters that we think are clinically relevant in terms of choice of anesthetic agent, dose, duration, and frequency of exposure. These animals are being followed longitudinally and will be evaluated for normal social, cognitive, and emotional development.

This report describes the socioemotional behavior evaluated at 6 months using the human intruder task. Designed to be similar to the task used for assessing dispositional anxiety and behavioral inhibition in children,[27,28] this task is well established in the primate literature as a robust test for assessing emotional reactivity based on the salience of the social threat presented by the intruder, is widely used for assessing emotional dysregulation in nonhuman primates,[29] and can be used longitudinally to examine the development of emotional behavior.[30] In addition, it is a robust task for detecting differences in emotional behavior according to genotype (*e.g.*, 5HTTLPR [serotonin transporter polymorphism] and CRHR1 [corticotrophin-releasing hormone receptor 1]), early life adversity, and after temporal lobe lesions in nonhuman primates.[31–38] Because species-typical emotional expression is not well organized on this task until after 4 months of age in macaques,[36,38,39] we tested subjects at 6 months of age, 5 months after their last anesthesia exposure. The human intruder task is able to discriminate dysfunction of specific neural systems including amygdala, orbitofrontal cortex, and hippocampus.[31,32,34,37] Thus, the pattern of emotional behavior of anesthesia-exposed monkeys could indicate the source of an underlying neurobiological impairment.

Materials and Methods

Subjects

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Emory University/Yerkes National Primate Research Center (Atlanta, Georgia; protocol 2002744). Twenty newborn rhesus monkeys (*Macaca mulatta*) of both sexes were born by vaginal delivery between April 2012 and May 2012 or between March 2013 and June 2013 with no veterinary intervention at the Field Station of Yerkes National Primate Research Center. Monkeys were drawn from two different available breeding compounds (A and B). Compounds were surveyed for new neonatal monkeys daily in the morning; the first day, a new neonatal monkey was identified was designated postnatal day 0. Neither monkeys born to the alpha (socially dominant) matriline in a group nor monkeys born to first-time mothers or to mothers with a history of poor maternal behavior were included in the study. This was done to avoid disrupting the stability of the large social groups through interactions with the alpha matriline and to avoid subjecting infant monkeys to anesthetic (or control) treatment that might not be able to continue in the study due to deficient maternal care. Infant monkeys were then assigned to anesthetic (five male and five female) or control (five male and five female) conditions matched for sex and weight to the extent possible. Eligible infants of sufficient size were assigned to groups as they were born, controlling for equal numbers in each cell, and matching for weight or age of the

first procedure (anesthesia exposures could only take place on certain days of the week) until the groups were filled. This yielded six female and four male infants in 2012 and four female and six male infants in 2013. Other than these considerations, assignment was random. An *a priori* power analysis revealed that 10 animals per group would give 80% power to detect an effect of Cohen $d = 1.156$ in a one-tailed t test, hypothesizing that early anesthesia exposure would result in cognitive impairment, corresponding to a partial η^2 of 0.25.

General Protocol for Access to Neonatal Monkeys

The mother and infant were guided from the home compound into a transport box the morning of the procedure (control or anesthesia) and brought to the nearby research building where they were transferred into a standard primate cage (24" × 28" × 30") in a housing room with other monkeys. The infant was removed from the mother and brought to the adjacent procedure room. The infant was weighed and a brief neurological test battery (Infant Neurobehavioral Assessment Scale [INAS], adapted from the study by Schneider and Suomi[4]) was conducted. At that point, the infant was either held gently in a blanket for 20 min and then returned to the mother (control) or mask induction with sevoflurane in 100% oxygen was initiated (anesthesia). This procedure was repeated three times for each monkey: first between postnatal days 6 and 10 and then again 14 and 28 days later. This control procedure equated the time the infant was conscious and separated from its mother in the two groups, avoiding prolonged maternal separation in the control infants which could have adverse effects of its own. The mother and infant remained in the colony room adjacent to the procedure room in the research building overnight and were returned to the home compound the next morning.

Anesthesia Protocol

A soft plastic mask was placed over the infant monkey's nose and mouth to administer sevoflurane in 100% oxygen at a flow rate of approximately 1 l/min. The initial vaporizer setting was 2% and was increased gradually to effect. When muscle tone was lost, the larynx was visualized with a laryngoscope (Miller Blade 00; Ace Vet Supplies, United Kingdom), and lidocaine 2% was sprayed onto the laryngeal folds. The monkey was intubated with a 1.5- to 2.5-mm-diameter uncuffed silicone endotracheal tube. Successful intubation was verified by capnograph trace. Intubation was unsuccessful in 6 of the 60 anesthetic procedures, and in these cases, anesthesia was delivered by mask only. At this point, the vaporizer setting was decreased to 2.5% and the air–oxygen mixture adjusted to an F_{IO_2} of approximately 0.3. Monitoring included capnography, agent analysis for sevoflurane and oxygen concentration (inspired and expired), pulse oximetry, rectal temperature, and indirect blood pressure (approximately mean of systolic and diastolic) *via* a cuff placed on the upper arm, and Doppler probe (Huntleigh Vettex Duo, United Kingdom) on the radial artery at the wrist. These parameters were recorded every 5 min during the anesthetic procedure, except blood pressure, which was measured every 10 min. Body temperature was maintained by a hot air blanket (3M; Bair Hugger, USA) placed over the infant. Venous blood gases were determined every 45 to 60 min during the procedure (Radiometer ABL80, USA). Intravenous fluids (lactated Ringer's solution) were given at a rate of 3 ml kg^{-1} h^{-1} during the procedure. If venous access was poor, blood gases were determined intermittently by percutaneous puncture, and fluids were given by subcutaneous bolus. The level of anesthesia was adjusted based on reaction to a calibrated pressure stimulus delivered between the third and fourth metatarsals on the foot, at a final pressure of 5 N reached at a rate of 2 N/s (Topcat Metrology Ltd., United Kingdom). If there was no motor response (limb withdrawal or increase in motor tone) and heart rate and respiration rate changed less than 10%, the vaporizer setting was left unchanged. If there was a motor response or the heart rate or respiration rate increased by more than 10%, the vaporizer setting was increased by 0.25%. If there was no motor response and no change in heart rate or respiration rate for two consecutive stimuli applied 15 min apart, the vaporizer setting was decreased 0.25%. In this way, we aimed to equate depth of anesthesia across subjects and anesthetic exposures. The duration of the anesthesia was 4 h after induction. At the end of this time, sevoflurane was terminated and the monkey was extubated when jaw tone increased and/or return of palpebral reflex was observed (*i.e.*, just before return of swallow reflex), usually within 2 min of termination of sevoflurane. Supplemental oxygen was provided by mask during the first few minutes postextubation if the infant did not maintain satisfactory blood oxygenation (pulse oximeter) breathing room air. Each infant was held by an experimenter wrapped in a blanket and observed for 20-min postextubation and then returned to the mother provided the infant was alert and no dyspnea or other overt physiological impairments were observed. Physiological measures during anesthesia were consistent with normal physiology (table 1).

Human Intruder Paradigm

At approximately 6 months of age, monkeys were transported to a novel testing room and then transferred to a stainless steel cage (53 cm × 53 cm × 55 cm) with one side made of clear Lexan plastic for video recording. The human intruder paradigm lasted 30 min and consisted of three conditions (Alone, Profile, and Stare) presented in the same order for all animals. First, the monkey remained alone in the cage for 9 min (Alone) to acclimate to the environment and obtain a baseline level of behavior. Then, the intruder (experimenter wearing a human rubber mask) entered the room and sat 2 m from the test cage while presenting his/her profile to the animal for 9 min (Profile condition). The intruder then left the room while the animal remained in the cage alone for a 3-min period, after which the

Table 1. Physiological Measures during Anesthesia

	First Anesthesia (P6-10)		Second Anesthesia (14 Days after First)		Third Anesthesia (28 Days after First)	
	Mean	SD	Mean	SD	Mean	SD
End-tidal carbon dioxide (mmHg)	37.55	3.54	39.72	3.18	37.72	1.78
Respiration rate (breaths/min)	52.53	11.31	45.19	7.91	43.07	10.40
Oxygen saturation of hemoglobin (%)	96.76	1.53	97.22	1.71	98.48	1.17
Pulse rate (beats/min)	152.57	22.24	158.86	15.39	160.05	18.79
Rectal temperature (°C)	37.09	0.38	37.19	0.30	37.11	0.33
Inspired sevoflurane (%)	2.48	0.17	2.61	0.16	2.66	0.27
Expired sevoflurane (%)	2.46	0.15	2.59	0.15	2.64	0.28
Blood pressure (mmHg)	56.37	19.76	52.09	17.55	55.43	13.99
Blood pH	7.38	0.03	7.39	0.05	7.41	0.03

P6-10 = postnatal days 6 to 10.

intruder reentered the room and made direct eye contact with the animal for 9 min (Stare condition). Emotional reactivity to the intruder was assessed *via* videotape recording for later coding using the Observer XT 10 software (Noldus Inc., The Netherlands) and a detailed ethogram (table 2). Three experimenters coded all of the videotapes but had a high degree of interrater reliability Cohen's $\kappa = 0.86$ and an average intrarater reliability of Cohen's $\kappa = 0.97$.

Statistics

Infant Neurobehavioral Assessment Scale. The temperament scale from the modified INAS test was analyzed using a multifactor ANOVA with group (control, anesthesia) and temperament item as the between-subjects factors and age (P7, P21, and P35) as the repeated measure.

Human Intruder Paradigm. One control male was not tested at this age due to illness, not related to the study, and thus was excluded from all behavioral analyses. Preliminary analyses were first performed to identify the outliers and determine the normality of the data. Interquartile range (IQR) was used to determine outliers, if an animals' behavior was more than 1.5 IQR above the third quartile or below the first quartile, then they were excluded from the analysis of that specific behavior. Thus, one anesthesia male was excluded from all behavioral analyses because the animal remained frozen throughout the test without performing any other behavioral activity (see Supplemental Digital Content 1, http://links.lww.com/ALN/B197, Results). In addition, one control male was found to be an outlier solely on anxiety behaviors (2.5 IQR above the third quartile) and thus was excluded from data analyses for this anxiety behavior only. Therefore, behavior analyses of vocalizations, freezing, and hostility included nine control monkeys (males = 4; females = 5) and nine anesthesia monkeys (males = 4; females = 5), whereas analyses of anxious behaviors included eight control monkeys (males = 3; females = 5) and nine anesthesia monkeys (see Supplemental Digital Content 1, http://links.lww.com/ALN/B197, Results).

Table 2. Behavioral Ethogram

Category and Specific Behavior	Measurement	Brief Definition
Vocalization behaviors	Cumulative frequency	
Coo	Frequency	Clear soft, moderate in pitch and intensity, usually "oooooh" sounding
Scream	Frequency	High-pitch, high-intensity screech or loud chirp
Fearful defensive behaviors		
Freeze	Duration	Rigid, tense, motionless posture except slight head movement
Hostile defensive behaviors	Cumulative Frequency	
Threat bark	Frequency	Low-pitch, high-intensity, rasping, guttural
Threat (facial expression)	Frequency	Any of the following: open mouth (no teeth exposed), head bobbing, or ear flapping
Cage aggression	Frequency	Vigorously slaps, shakes, or slams body against cage
Lunge	Frequency	A quick, jerky movement toward the intruder
Anxious behaviors	Cumulative frequency	
Scratch	Frequency	Rapid scratching of body with hands or feet
Body shake	Frequency	Whole body or just head and shoulder region shakes
Tooth grind	Frequency*	Repetitive, audible rubbing of upper and lower teeth
Yawn	Frequency	Open mouth widely, exposing teeth

List of all behaviors scored, how they are measured, and a brief definition.

* Behavior for which total duration was also measured.

Normality of the data was determined using the Kolmogorov–Smirnov tests. Only anxious behaviors were not normally distributed and were transformed using a natural log plus constant (1) to obtain normality. Although there were no hypotheses regarding sex differences in effect of anesthesia exposure, we are underpowered to detect sex differences, and sex differences in the human intruder task are not found at this age,[30,38] exploratory repeated-measures ANOVAs with group (control, anesthesia) and sex as between-subjects factors and condition (Alone, Profile, and Stare) as the within-subjects factor were initially conducted to determine whether any effects of sex were present in the data. Because no significant effects of sex were found, final analyses that examined anesthesia effects on emotional reactivity were repeated-measures ANOVAs with group as a between-subjects factor and condition as a within-subjects repeated measure. All analyses were conducted with SPSS 16 for Windows (IBM Corporation, USA), and significance level was set at P value less than 0.05. Effect sizes were calculated using partial η^2 ($_p\eta^2$) and Cohen's d (d). Partial η^2 is the ratio of variance accounted for by an effect plus its associated error of variance within an ANOVA and can be interpreted like R^2 by moving the decimal point two places to the right, then interpreting the value as a percentage of variance associated with the effect. Cohen's d indicates the standardized difference between two means, where values of 0.2, 0.5, and 0.8 are considered to indicate a small, medium, and large effect size, respectively.

Results

Data from the INAS test revealed no group differences on temperament items (group: $P = 0.58$, $_p\eta^2 = 0.004$, $d = 0.26$) or group interactions (group × age: $P = 0.30$, $_p\eta^2 = 0.02$, $d = 0.52$; group × temperament item: $P = 0.58$, $_p\eta^2 = 0.004$, $d = 0.25$; and age × group × temperament item: $P = 0.68$, $_p\eta^2 = 0.03$, $d = 0.38$), indicating that before each anesthesia exposure, infants in the anesthesia group did not differ from controls in their level of anxiety, their reaction to maternal separation, and/or induction of anesthesia. However, there was a significant interaction for age × temperament item ($P = 0.004$, $_p\eta^2 = 0.12$, $d = 0.86$; see also Supplemental Digital Content 1, http://links.lww.com/ALN/B197, Results): infants from both groups exhibited species-typical pattern of increased scores for vocalizations, fear, and irritability with age and decreased consolability scores with age (linear function: $P = 0.001$, $_p\eta^2 = 0.21$, $d = 1.19$). Additional statistical results from the INAS test are given in Supplemental Digital Content 1, http://links.lww.com/ALN/B197, Results.

The anesthesia group exhibited sparing of some behaviors and increased expression of others in the human intruder task. When placed alone in a novel environment or faced with a direct threat, infant monkeys emit vocalizations in an attempt to reconnect with their mother. This pattern of vocalizations was observed in both controls and anesthesia animals, such that all infants emitted significantly more vocalizations in the Alone and Stare conditions compared with the Profile (condition: $P = 9.65 \times 10^{-10}$, $_p\eta^2 = 0.73$, $d = 3.26$) with no differences between controls and anesthesia monkeys (group: $P = 0.58$, $_p\eta^2 = 0.02$, $d = 0.28$; condition × group: $P = 0.15$, $_p\eta^2 = 0.11$, $d = 0.7$; fig. 1A; Supplemental Digital Content 1, http://links.lww.com/ALN/B197, Results). When faced with the mild threat of the intruder's profile, both groups displayed the species-typical fearful defensive behavior of increased freezing (condition: $P = 3.04 \times 10^{-12}$, $_p\eta^2 = 0.81$, $d = 4.12$; fig. 1B), with no differences found between controls and anesthesia animals (group: $P = 0.27$, $_p\eta^2 = 0.07$, $d = 0.57$; condition × group: $P = 0.57$, $_p\eta^2 = 0.03$, $d = 0.37$). The most salient threat to a monkey occurs when the intruder makes direct eye contact. During this Stare condition, both groups exhibited increased hostile behaviors (condition: $P = 4.49 \times 10^{-10}$, $_p\eta^2 = 0.74$, $d = 3.37$). Expression of hostile behaviors was comparable between infants with early anesthesia exposure and controls although not statistically significant the $_p\eta^2$ effect size accounted for 20% of the variance (group: $P = 0.06$, $_p\eta^2 = 0.20$, $d = 0.99$; fig. 1C). There was no significant interaction for hostile behaviors (condition × group: $P = 0.61$, $_p\eta^2 = 0.03$, $d = 0.31$). The Stare condition also evoked an increase in anxious behavior expression in both groups (condition: $P = 2.27 \times 10^{-14}$, $_p\eta^2 = 0.87$, $d = 5.34$; fig. 1D), and anesthesia infants expressed significantly more anxious behaviors overall as compared with controls with large effect sizes, accounting for 33% of the variance (group: $P = 0.016$, $_p\eta^2 = 0.33$, $d = 1.41$; fig. 1D). The condition by group interaction was not significant ($P = 0.41$, $_p\eta^2 = 0.06$, $d = 0.49$). Statistical results for these comparisons including the two subjects that were statistical outliers are included in Supplemental Digital Content 1, http://links.lww.com/ALN/B197, Results.

Discussion

Infant rhesus monkeys who received multiple exposures to sevoflurane during the first month of life exhibited increased anxiety 5 months after exposure. These results demonstrate that early anesthesia exposure, in the absence of a surgical procedure, comorbidities, or psychosocial stress associated with illness or the need for a surgical procedure, causes alterations in emotional behavior. These results are consistent with previous reports demonstrating increased anxiety after damage to the limbic system.[31,32,34,37]

Evidence from rodents and monkeys have shown that either single or multiple exposures to volatile anesthesia early in life has a neurotoxic effect on the developing brain,[7–21] targeting both neurons and glia, with oligodendrocytes engaged in myelinogenesis being particularly vulnerable.[7,12–14,16,20,21] Although apoptosis is widespread in the brain, cell death rates in areas implicated in emotional behavior such as the amygdala, prefrontal cortex, and hippocampus vary by anesthesia type, duration of exposure, and brain region of interest. For example a single exposure to sevoflurane resulted in a 5- to 29-fold increase in neurodegeneration in different

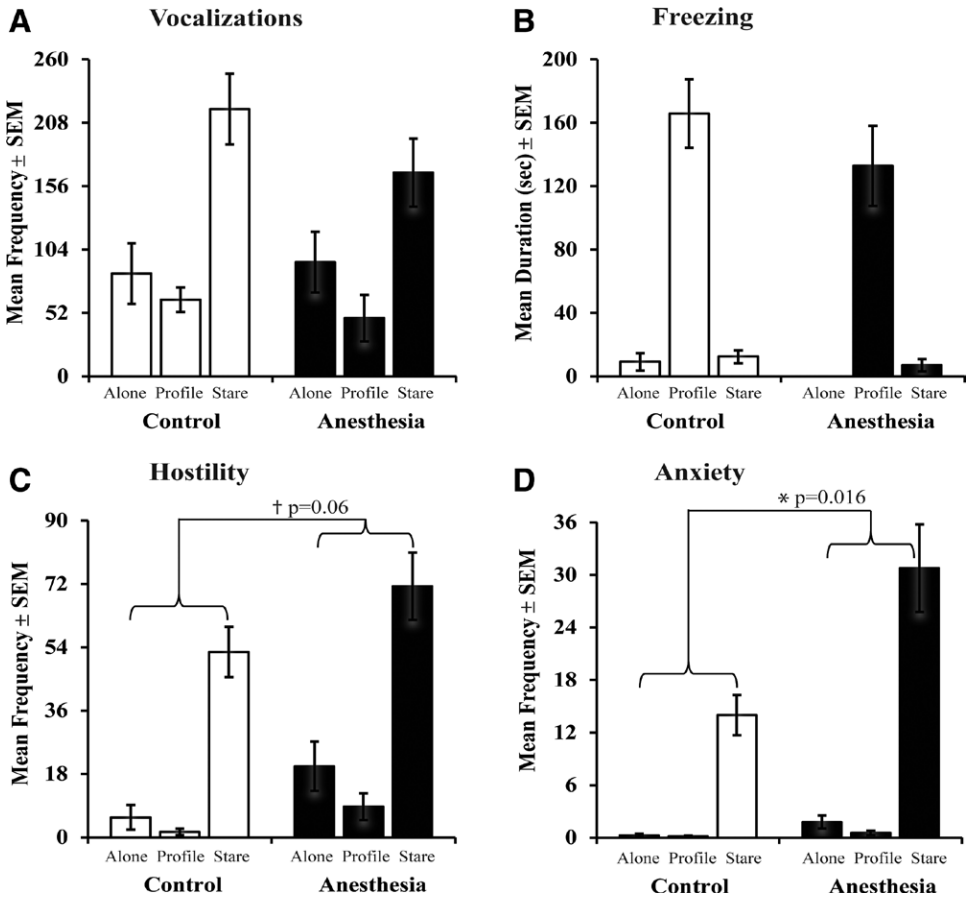

Fig. 1. Emotional behavior responses on the human intruder paradigm: mean ± SEM vocalizations (*A*), freezing (*B*), hostility (*C*), and anxiety (*D*). Anxiety behavior expression was transformed (LN x + 1) for data analysis, and nontransformed data are graphed. Control animals are represented by *open bars* and anesthesia animals are represented by *closed bars*. There was a significant condition effect for all four behaviors. *A significant group difference ($P < 0.05$). †A nonsignificant group difference ($P = 0.06$) with a large effect size.

parts of the hippocampus.[12,22,23] Considering that adult hippocampal damage in humans and neonatal hippocampal lesions in monkeys result in increased anxiety expression to a stressor,[16,37,40] it is possible that anesthetic-induced damage to the developing hippocampus causes the anxious phenotype seen in our anesthesia-exposed animals.

The major strength of this study is its ability to separate anesthesia exposure from surgical procedures, which is a potential complication in the studies conducted in children. Our results confirm that multiple anesthesia exposures alone result in emotional behavior changes. As mentioned, these changes may reflect adverse effects of early anesthesia exposure on hippocampal function, to which memory deficits after early anesthesia exposure in rodents have also been attributed.[7,10,12,26,41] However, the lack of surgical manipulation can also be seen as a limitation of this study, in terms of reproducing the situation in which children are exposed to anesthesia. Surgical manipulation would induce postoperative pain, inflammation, and the need for postoperative medications, all factors that may affect postoperative behavior and that are not addressed in our model. Nevertheless, we are able to exclude these as possible explanations for our findings.

To date, the majority of research has focused on learning and memory impairments in rodents after early anesthesia exposure, with few exceptions.[12,27,28] However, there are limitations to using rodents to model human behavior,[42,43] whereas nonhuman primates exhibit many similarities to humans. Nonhuman primates have similar brain morphology, genetics, endocrine systems, live in complex social groups, and use visual cues to extract socioemotional information from their environment,[30,44] perhaps making them an ideal animal model to examine emotional changes after early exposure to anesthesia. The direct measure of emotional behavior using the human intruder paradigm in our nonhuman primate model might be considered a major strength of the current study, in contrast to studies of human children in which negative behavior changes are typically measured by behavioral surveys completed by the parents that may be insensitive to subtle changes in emotional behavior.[6,29] Future studies in human children might use related paradigms to assess emotional behavior.

A minor limitation is the sample size in our study. Although large for a nonhuman primate study, having only five males and five females in each group limits our statistical power to detect sex differences. Our study design was

powered for detection of group differences irrespective of sex, and we concluded that any indication of sex differences could be followed up in future work designed specifically to address hypotheses about sex differences. Moreover, sex differences during the intruder task have not been detected when gonadal hormones are low in circulation,[30,38] as is the case in the current study of 6-month-old infant monkeys. The presence of group differences with substantial effect sizes that do not reach conventional criteria for statistical significance, such as the effect of anesthesia condition on hostility-related behavior, suggests that we were underpowered to detect more subtle effects of anesthesia exposure that may be present.

Our results also demonstrate that alterations in emotional behavior persist up to 5 months after anesthesia exposure suggesting long-term effects. Exactly how long these changes in emotional behavior will persist in our anesthesia-exposed monkeys is unclear. We will continue to follow these animals behaviorally to fully characterize the length of time that these changes persist and whether changes in emotional behavior resolve over time, suggesting a transitory change or resilience. It can also be considered that we are able to detect adverse behavioral effects 5 months after the last exposure to anesthesia, whereas cognitive impairments that have been identified in children after repeated anesthesia exposure are ascertained at school age, years after the last exposure to anesthesia.[1,31–38] Thus, future prospective studies in humans may be able to use tests of emotional behavior to identify individuals at risk of later learning disabilities or other cognitive impairments. Considering that most pediatric surgeries are nonelective,[36,38,39,45,46] future studies can use this primate model to develop a new anesthetic agent or prophylactic treatment to counteract the impact of anesthesia on behavior in children.

Conclusions

These findings are consistent with the view that exposure to anesthetic agents, specifically, may cause long-term alterations in central nervous system function that lead to abnormal cognitive and emotional behavior later in life. Monkeys in this study that were exposed to sevoflurane anesthesia three times in the first 6 weeks of life showed increased anxiety-related behaviors over 5 months later.

Clearly, when surgery is indicated in infants or young children for correction of serious health issues, the consequences of failing to perform the surgical procedure typically outweigh any possible risks of neurocognitive changes. Moreover, these data do not conclusively demonstrate a link between anesthetic exposure and altered emotional behavior or other cognitive deficits after surgery in humans. Nevertheless, they support attention to emotional behavior and management of anxiety as part of postsurgical management and monitoring of infants and children. Furthermore, additional work is required to identify the mechanisms by which anesthetics may cause long-term changes in central nervous system function that impact behavior, so that strategies can be identified to offset or prevent these changes while maintaining the essential beneficial effects of anesthesia that allow safe surgical interventions.

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Competing Interests

The authors declare no competing interests.

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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

"Fac-Simile" Confederate $10 Bill Advertising Odontunder by Dr. A. B. Cobb

From Richmond, Virginia, on September 2, 1861, the Confederate States of America released a $10 banknote. Both that original bill and the advertising "Fac-Simile" of it (*top*) feature the same images (from left to right) of John E. Ward, a "Wagon Load of Cotton," and corn gatherers. On the back of the ersatz banknote (*bottom*), there is an advertisement by Dr. Arthur B. Cobb of Buffalo, New York, touting that he obtunds (numbs) patients' gums with "Odontunder" (a portmanteau fusing Greek for "tooth" with Latin for "to blunt or dull"). By 1906 chemical analyses of Odontunder revealed that the proprietary local anesthetic mixture contained cocaine, carbolic acid (phenol), and resorcin (a "chemical cousin" of phenol). This facsimile banknote advertisement is part of the Wood Library-Museum's Ben Z. Swanson Collection. (Copyright © the American Society of Anesthesiologists, Inc.)

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