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Multiple sevoflurane exposures in infant monkeys do not impact the mother-infant bond

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Abstract

Exposure to general anesthesia during the postnatal period is associated with death of brain cells as well as long-term impairments in cognitive and emotional behavior in animal models. These models are critical for investigating mechanisms of pediatric anesthetic neurotoxicity as well as for testing potential strategies for preventing or mitigating this toxicity. Control conditions for anesthesia exposure involve separation of conscious infants from their mothers for variable periods of time, which could have its own effect on subsequent behavior because of stress to the mother and/or infant as a consequence of separation. We are conducting a long-term study of infant rhesus monkeys exposed three times for 4 hours each to sevoflurane anesthesia during the first six postnatal weeks, with a comparison condition of control infant monkeys that undergo brief maternal separations on the same schedule, to equate the period of time each infant is conscious and separated from its mother. Because mothers are separated from their infants longer for infants in the anesthesia condition, this could modify maternal behavior towards the infant, which may influence subsequent socioemotional behavior in the infants. In this study, we analyzed maternal behavior immediately after the first post-anesthesia (or control) reunion, as well as during reintroduction of the mother-infant pair to the larger social group 24 hours post-anesthesia or control separation, and found no differences between the conditions with mothers spending most

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of their time in contact with infants in all conditions analyzed. This indicates that the different durations of maternal separation in this study design do not impact the mother-infant bond, strengthening conclusions that subsequent differences in behavior between monkeys exposed to anesthesia compared to controls are a consequence of anesthesia exposure and not differential maternal behavior in the two conditions.

Keywords

maternal behavior; anesthesia; pediatric anesthetic neurotoxicity; macaque

1. Introduction

In 2012, the FDA, American Academy of Pediatrics, and International Anesthesia Research Society released a consensus statement acknowledging the growing evidence that general anesthetic (GA) exposure early in life poses an increased risk for learning and memory impairments later in life (Rappaport, et al., 2015). Although recent evidence has shown single, short anesthetic exposures to be safe in young children (Davidson, et al., 2015), there is a proportional increased risk of learning disabilities with each additional GA exposure (Wilder, et al., 2009; Flick et al., 2011). Therefore, multiple exposures to general anesthetics pose the greatest risk for cognitive impairments in children.

Animal studies have shown that behavioral changes can result from exposure to anesthesia alone in the absence of a disease state or complications arising from surgery. Early anesthesia exposure in rodents and nonhuman primates causes damage throughout the brain, including the prefrontal cortex, hippocampus, and amygdala (Jevtovic-Todorovic, et al., 2003; Yon, et al., 2005; Slikker et al., 2007; Satomoto, et al., 2009; Brambrink, et al., 2010; Istaphanous, et al., 2011; Amrock, et al., 2015; Liu et al., 2015; Paule, et al., 2011). These brain areas are important for normal cognitive functioning and socioemotional behaviors, both of which are impacted by exposures to GA in animal models (Satomoto, et al., 2009; Shen, et al., 2013; Raper, et al., 2015; Zou, et al., 2015). However, the control conditions of many animal studies involved long conscious maternal separation equivalent to the anesthetic exposure time.

Maternal separation can adversely affect brain and behavioral development (Rilling, et al., 2001; Sanchez, et al., 2001, 2005; Brenhouse & Andersen, 2011; Parr, et al., 2012). Importantly, separation of the infant from the mother is independently stressful to the infant as well as to the mother. In designs where the control condition is a separation of equal time from the mother (perhaps including exposure to anesthesia carrier gases), control infants consciously experience the separation longer than anesthetized infants, potentially inducing stress in the control condition that is not present in the anesthesia condition. Therefore, prolonged maternal separation in controls could confound or mask the impact of anesthesia exposure, especially on socioemotional behavior.

For this reason, in our recently published study on the effects of anesthesia exposure on socioemotional behavior in infant rhesus macaques (Raper, et al., 2015) we elected to equate the conscious experience of maternal separation between control and exposed infants. In that

study, infant monkeys were exposed to 4 hours of sevoflurane three times, beginning approximately at postnatal day 7 and then again 2 and 4 weeks later. Control infants experienced 20-minute maternal separation at the same age points, which equalized the amount of time the infants in either group were conscious and away from their mothers. Despite the same amount of conscious separation, infants in the exposed group showed abnormal amounts of anxiety and hostility in a social stress test at 6 months of age (Raper, et al., 2015). However, it is important to note that the mothers in the two conditions had different experiences that might have affected their behavior towards the exposed infants, which could have its own effects on subsequent socioemotional behavior in infants. Thus, in this report we analyzed the mother-infant interactions when the pairs were reunited after the first anesthesia or control separation, as well as when the pair was reintroduced into their social group.

2. Methods

2.1 Subjects

All procedures have been published in detail (Raper, et al., 2015). Briefly, subjects were twenty infants (10 male, 10 female) comprising two cohorts. An a priori power analysis revealed that 10 animals per group would give 80% power to detect an effect of Cohen's $d=1.156$ in a one-tailed t-test, hypothesizing that early anesthesia exposure would result in cognitive impairments. Cohort 1, born in the spring of 2012 comprised 6 female and 4 male infants; Cohort 2, born in 2013, comprised 4 female and 6 male infants. Infants were born without assistance to middle-ranking dams in either of two large (50-100 individuals, in multiple families) social groups. They were assigned to anesthetic (5 male and 5 female) or control (5 male and 5 female) conditions matched for sex and weight to the extent possible and assigned to groups as they were born, controlling for numbers in each cell, weight and age of first procedure (anesthesia exposures could only take place on certain days of the week) until the groups were filled. Other than these considerations, assignment was random. All procedures and care for the animals followed the NIH Guide for the Care and Use of Laboratory Animals and experimental protocols were approved by the Institutional Animal Care and Use Committee of Emory University.

2.2 General protocol for access to neonatal monkeys

The mother and infant were removed from their home compound the morning of the procedure (control or anesthesia) and transported to a nearby research building where they were housed in a standard primate cage (24" × 28" × 30") in a room with other monkeys. The infant was removed from the mother, brought to the adjacent procedure room, weighed and given a brief neurological test battery (Infant Neurobehavioral Assessment Scale [INAS], adapted from Schneider & Suomi, 1992). At that point, the infant was either held gently in a blanket for 20 minutes and then returned to the mother (control) or mask induced with sevoflurane in 100% oxygen (anesthesia). This procedure was given 3 times for each monkey: first between postnatal days 6-10, then again 14 and 28 days later (between postnatal days 20-24, and 34-38, respectively). 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.

2.3 Anesthesia protocol

Details of the anesthetic protocol and physiology during anesthesia are given in Raper et al. (2015). Briefly, anesthesia was induced by mask (sevoflurane in 100% oxygen, initial concentration 2% and increased to effect) and once muscle tone was lost, the infant monkey was intubated and oxygen concentration was reduced (FiO_2 0.3, balanced with medical air) and sevoflurane set to 2.5% on the vaporizer. In 6 of the 30 anesthetic procedures intubation was unsuccessful and in these cases anesthesia was delivered by mask only. The duration of the anesthesia was 4 hours after induction. Physiological monitoring included capnography, agent analysis for sevoflurane and oxygen concentration, pulse oximetry, rectal temperature, and indirect blood pressure via a cuff placed on the upper arm and Doppler probe (Huntleigh Vettex Duo) on the radial artery at the wrist. The level of anesthesia was adjusted based on reaction to a calibrated pressure stimulus delivered between the third and fourth metatarsals on the foot. Post-extubation each infant was wrapped in a blanket, held by an experimenter and observed for 20 minutes, 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.

2.4 Mother-infant interactions

2.4.1 Reunions after anesthesia or control procedure—Prior to the first procedure, mother-infant pairs had not been separated for any experimental or clinical reasons, so the reunion after this first procedure was critical to ensure that the mother would not reject the infant. Thus, mother-infant pairs were monitored initially in person for 10 minutes, immediately followed by videotaping for a minimum of 30 minutes after the first anesthetic or control procedure. After the first anesthetic or control procedure, mother-infant pairs were routinely accessed and briefly separated (2-5 minutes) for weekly health and weight monitoring of the infant. Therefore, because the mother-infant pair was familiar with separation and reunion procedures following the second and third anesthesia or control procedures, these were not recorded or analyzed.

2.4.2 Reintroduction of mother-infant pair into social group—Twenty-four hours after the anesthetic or control procedure, mother-infant pairs were returned to their social group. Reintroduction of the pair into their social group after each anesthetic or control procedure was videotaped for 30 minutes.

Maternal behavior was assessed using the videotapes from reunions and social group reintroductions, which were coded using the Observer XT 10 software (Noldus Inc., Netherlands) and a detailed ethogram (Table 1). Three experimenters blinded to the treatment condition coded all of the videotapes, with average inter-rater reliability Cohen's Kappa = .87 and intra-rater reliability of Cohen's Kappa = .95.

2.5 Statistical Analyses

2.5.1 Reunion after first anesthesia or control procedure—Six control animals were unable to be videotaped after their control procedure due to the mother requiring clinical care or physical exam for a minor injury (C7m, C8m, C9m, C10m), the infant needing treatment for diarrhea (C4f), or camera malfunction (C3f). Therefore, 14 animals

were included in the analyses (controls: males = 1, females = 3; anesthesia: males = 5, females = 5; see Table 2). Although animals were counterbalanced by birthing cohort, preliminary analyses were conducted with Cohort as an additional between-subjects factor. One-way ANOVAs were conducted with Group (control, anesthesia) and Cohort (cohort 1, cohort 2) as between subjects factors for each behavior. Because no significant effects of Cohort were found, final analyses that examined anesthesia effects on mother-infant interactions were One-way ANOVAs with Group (control, anesthesia) as between-subjects factor.

2.5.2 Reintroduction of mother-infant pair into social group—The first reintroduction was unable to be done on the same schedule as for other monkeys for one control female (C4f) due to her being treated for diarrhea and housed with her mother in the clinic during that time. Therefore, 19 animals were included in the analyses (controls: males = 5, females = 4; anesthesia: males = 5, females = 5; see Table 2). Preliminary analyses were conducted with Cohort as an additional between-subjects factor. Repeated measures ANOVAs were conducted for the mother-infant pair returning to their social group with Group and Cohort as between-subjects factors and Time (Reintroduction 1, 2, & 3) as the within-subjects factor with repeating measures. Because no significant effects of Cohort were found, final analyses were repeated measures ANOVAs with Group between-subjects factor and Time as the within-subjects factor with repeating measures.

To examine the relative role that the mothers and infants play in maintaining a close distance to each other (i.e. full body contact and proximity), the Hinde index (Hinde & Atkinson, 1970) and Brown index (Brown, 2001) were calculated. Leaving was defined as moving beyond 3 meters away. The Hinde index was calculated by subtracting the percentage of leaving events initiated by the infant (infant leaving / infant + mother leaving) from the percentage of approaches (i.e. contact and proximity) the infant made toward the mother (infant approaches / infant + mother approaches). A positive value indicates that the infant is mainly responsible for a greater proportion of approaches made with the mother than leaving the mother. The Brown index is calculated as a ratio of approaches and leaving the mother initiated by infants, to all of the approaches and leaving initiated by both mothers and infants. The Brown index assesses whether the mother or the infant is responsible for the majority of changes in maintaining close distance (contact and proximity). The Hinde and Brown indices were analyzed using repeated measures ANOVAs with Group between-subjects factor and Time as the within-subjects factor with repeating measures.

All analyses were conducted with SPSS 22 for Windows and significance level was set at $p < 0.05$. Effect sizes were calculated using partial eta squared (η^2). Partial eta squared is the proportion of variance accounted for by an effect plus its associated error of variance within an ANOVA model, and thus is bounded by 0 and 1.

3. Results

3.1 Reunion after first anesthesia or control procedure

All mothers readily retrieved their infants after the first anesthesia or control procedure. Additionally, in the reunions that were recorded, mothers spent nearly all of their time in full

body contact with their infant with no significant difference between the anesthesia and control groups ($F[1,13]=0.33$, $p=0.57$, $p\eta^2=.03$; see Figure 1A). Lastly, mothers spent the majority of their time engaged in maternal behaviors (grooming and cradling). Although mothers of anesthesia infants spent slightly more time engaged in maternal behaviors there was no significant difference between the groups ($F[1,13]=3.33$, $p=0.09$, $p\eta^2=.22$; see Figure 1B).

3.2 Reintroduction of mother-infant pair into social group

All mother-infant pairs were successfully reintroduced back into their social groups and the mothers were able to reintegrate into the social hierarchy. During the first few months of life, rhesus infants spend the majority of their time in close contact with their mother being groomed and cradled; by approximately 3 months old, infants spend significant amounts of time beyond two feet away from their mother (Hansen, 1966; Hinde & Spencer-Booth, 1967). The current data were consistent with these previous reports. Overall, the mother-infant pairs spent most of their time in full body contact after every social group reintroduction (Time: $F[2,34]=0.82$, $p=0.45$, $p\eta^2=.05$) with no interactions or group differences (Group $\times$ Time: $F[2,34]=0.12$, $p=0.88$, $p\eta^2=.01$; Group: $F[1,17]=0.14$, $p=0.71$, $p\eta^2=.01$; see Figure 1C). By contrast, infants spent a small percentage of time only within arm's reach/proximity of the mother across the three reintroductions (Controls = 14.36 ± 2.87 ; Anesthesia = 16.17 ± 2.73). There were no effects of time, group, nor interactions for proximity to the mother (Time: $F[2,34]=0.79$, $p=0.46$, $p\eta^2=.04$; Group: $F[1,17]=0.21$, $p=0.65$, $p\eta^2=.01$; Group $\times$ Time: $F[2,34]=1.07$, $p=0.35$, $p\eta^2=.06$). Similarly, they spent a small percentage of time beyond 3 meters/away for their mother (Controls = 7.72 ± 1.56 ; Anesthesia = 7.52 ± 1.48). There were no effects of time, group, nor interactions for time away from the mother (Time: $F[2,34]=1.17$, $p=0.32$, $p\eta^2=.07$; Group: $F[1,17]=0.01$, $p=0.93$, $p\eta^2=.001$; Group $\times$ Time: $F[2,34]=0.44$, $p=0.64$, $p\eta^2=.03$). Therefore, mother-infant pairs spent the majority of their time in full body contact, with mothers spending similar amounts of time engaged in maternal behaviors across all three introductions (Time: $F[2,34]=1.68$, $p=0.2$, $p\eta^2=.09$). Although mothers of anesthesia infants engaged in numerically more maternal behaviors during the first reintroduction, there were no significant interactions or group effects (Time $\times$ Group: $F[2,34]=0.39$, $p=0.68$, $p\eta^2=.02$; Group: $F[1,17]=1.36$, $p=0.26$, $p\eta^2=.07$; see Figure 1D).

The Hinde index revealed that the maintenance of close distance between the mother and infant was positive and did not change across the three reintroductions (Time: $F[2,34]=0.91$, $p=0.41$, $p\eta^2=.05$). Likewise, there was no significant interaction or group differences (Group $\times$ Time: $F[2,34]=0.35$, $p=0.71$, $p\eta^2=.02$; Group: $F[1,17]=0.10$, $p=0.75$, $p\eta^2=.01$; see Figure 1E). Therefore, both anesthesia and control infants equally initiated more approaches (contact and proximity) with the mother than leaving beyond 3 meters/away of the mother. The Brown index indicated that infants were generally responsible for the majority of changes in maintaining a close distance to the mother, and this did not change across the three reintroductions (Time: $F[2,34]=0.47$, $p=0.63$, $p\eta^2=.03$), and there were no interaction or group differences (Group $\times$ Time: $F[2,34]=0.40$, $p=0.67$, $p\eta^2=.02$; Group: $F[1,17]=1.05$, $p=0.32$, $p\eta^2=.06$; see Figure 1F).

4. Discussion

Maternal behavior was not impacted by multiple exposures to sevoflurane during the first month of life. As has been previously shown, rhesus mother-infant pairs spend the majority of their time in close contact during the first few months of life (Hansen, 1966; Hinde & Spencer-Booth, 1967). Additionally, the Hinde and Brown indices indicated that both anesthesia and control infants were initiated more approaches (contact and proximity) than leaving beyond 3 meters/away of their mother and were responsible for the majority of changes in maintaining a close distance to the mother. To our knowledge this is the first report specifically comparing mother-infant interactions after a prolonged exposure of the infant to anesthesia with a brief control separation. Maternal behaviors toward the infant were the same whether the infant had undergone three long (4 hours of infant anesthesia exposure) or three brief (20 minutes of infant control procedure) maternal separations. Thus, we conclude the mother-infant bond was not impacted by the longer separations required for exposure of the infants to anesthesia.

Maternal separation has been shown to disturb mother-infant interactions as well as the normal development of the brain and behavior of infant rodents and nonhuman primates (Sanchez et al, 2001; Brenhouse & Andersen, 2011). Brief repeated maternal separations for 90 days altered the time that rhesus monkey mother-infant pairs spent in close contact, and was associated with increased anxiety as juveniles, as measured by increased acoustic startle and cortisol stress response (Sanchez, et al, 2005), as well as decreased hippocampal activity in adulthood (Parr et al, 2012). The absence of differences in mother-infant interactions between anesthesia and controls, supports our previous finding of increased anxiety at 6 months of age in anesthesia infants being the result of anesthesia exposure and not maternal separation (Raper, et al, 2015). Future studies are needed to assess whether similar alterations in anxiety, but not mother-infant interactions, would be found relative to infants that never undergo maternal separation or those that underwent maternal separation equivalent to the anesthesia exposure time. Despite this logical possibility, the key finding that mother-infant interactions do not differ between control and anesthesia conditions in our current study remains.

Animal studies have demonstrated that early exposure to GA has a widespread neurotoxic effect on the developing brain (Jevtovic-Todorovic, et al, 2003; Yon, et al, 2005; Slikker et al, 2007; Satomoto, et al, 2009; Brambrink, et al, 2010; Istaphanous, et al, 2011; Paule, et al, 2011; Amrock, et al, 2015; Joksovic, et al, 2015; Liu et al, 2015), particularly in areas important for cognition and socioemotional behavior, such as the prefrontal cortex, hippocampus, and amygdala. In monkeys, neonatal amygdala or orbitofrontal cortex lesions alter mother-infant interactions (Bauman, et al, 2004; Goursaud & Bachevalier, 2007; Raper, et al, 2014; but see Goursaud, et al, 2014). Thus, our current study demonstrates that the mother-infant bond is not affected by anesthesia exposure despite the potential for our anesthesia protocol to have impacted the normal development of the amygdala and frontal cortex.

Our studies of cognition and socioemotional behavior in this population of monkeys are ongoing but we have already reported elevated anxiety-related behaviors in monkeys

exposed to sevoflurane as infants, as compared to separated controls, when their emotional response to an acute mild stressor (an unfamiliar human) was tested at 6 months of age (Raper, et al, 2015). Our analysis of maternal behavior in our study population reinforces our conclusions that altered emotional behavior is attributable to a long-term effect of anesthesia exposure, rather than some other difference between anesthesia and control conditions related to the logistics of the different treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Animal studies demonstrate brain and behavioral changes result from exposure to anesthesia early in life
- Maternal separation can also alter brain and behavioral development, which could confound or mask the impact of early anesthesia exposure
- Maternal behaviors were similar between infants undergoing three long anesthesia exposures or three brief maternal separations
- Mother-infant bond was not impacted by multiple exposures to anesthesia during early infancy

Figure 1.

Mother infant interactions:

Mean $\pm$ SEM of Percent time in close contact (A) and maternal behavior (B) after the first anesthesia or control procedure. Mean $\pm$ SEM of the mothers response after each reintroduction to the social group: Percent time in close contact (C), maternal behavior (D), Hinde index (E), and Brown index (F). Control animals are represented by open bars and anesthesia animals are represented by closed bars. There were no significant effects of group nor interactions for any behaviors.

Table 1**Behavioral Ethogram**

Category & Behavior	Measurement	Description
Distance to Mother		
Contact	Duration	Maintains touching of its' whole body with the mother
Proximity	Duration	Stays within arm's reach of the mother
Away	Duration	Goes beyond 3 meters distance from the mother
Maternal Behaviors	Cumulative Duration	
Cradle	Duration	Mother holds the infant against her ventrum with arm(s) wrapped around the infant
Groom	Duration	Mother combs through the hair of her infant
ⁿ Reject Infant	Frequency	Mother prevents the infant from contact or nursing
ⁿ Abuse Infant	Frequency	Mother dragged, threw, stepped or sat on the infant

Categories and list of all behaviors included in this study, how they were measured, and a brief definition.

ⁿ indicates that the behavior was not seen.

Table 2

Behavioral Assessments Summary

Behavioral Assessment	Group	Sex	Sample number
Reunion after anesthesia or control procedure	Controls	F	n=3
		M	n=1
	Anesthesia	F	n=5
		M	n=5
Reintroduction of mother-infant pair into their social group	Controls	F	n=4
		M	n=5
	Anesthesia	F	n=5
		M	n=5

List of behavioral assessments included in this study and the sex and number of animals included in each group for the statistical analyses.