Monkey in the Middle

Translational Studies of Pediatric Anesthetic Exposure

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A SEMINAL report by Jevtovic-Todorovic *et al.*[1] described neurotoxicity and long-term cognitive impairments after exposure to general anesthesia in infant (postnatal day 7) rats. This phenomenon has been observed repeatedly in multiple species and with a variety of different anesthetic drugs. It has also sparked parallel retrospective and prospective studies in humans that suggest that pediatric anesthesia might be associated with increased risk of adverse neurocognitive outcomes when exposure is repeated or prolonged.[2–9] The recent investigation by Coleman *et al.*[10] joins a growing number of studies investigating the impact of exposure to general anesthesia in infancy on neurobehavioral development in nonhuman primates. This highly translationally relevant model makes unique contributions to understanding the phenomena and potential mechanisms of long-term cognitive and behavioral changes after general anesthesia early in life.

"…work with nonhuman primates forms a critical middle ground for future investigations of...neurobehavioral function after… anesthesia exposure…"

There are a number of important advantages to studying this question in nonhuman primate models, not the least of which is the ability to study the effects of general anesthesia in the absence of surgery. Physiologic monitoring and support is much more feasible in an infant monkey than in an infant rodent. Thus, long-term effects of anesthesia exposure cannot easily be attributed to hypoxia, hypercapnia, and so forth. The stage of brain development at birth of a rhesus monkey is similar to that of a 6-month-old human infant,[11,12] whereas based on many neurobiologic markers, postnatal day 7 rats that are at peak vulnerability to anesthetic neurotoxicity[13] may more closely match the stage of brain development of a third-trimester human fetus. Nonhuman primates also have the capacity to perform more complex tasks and have a sophisticated social structure, like that of humans, allowing more translationally relevant tests of cognition and socioemotional behavior. Because puberty occurs between 3 and 5 yr of age in rhesus monkeys, it is possible to examine changes in behavior over the course of development that would be challenging to study in rodent models, in which sexual maturity occurs around the age of 6 weeks.

The primary outcomes in the study by Coleman *et al.*[10] are measures of early motor reflexes and emotional behavior in a number of different situations. They employ test batteries for monkeys that were developed to be analogous to behavioral testing procedures in humans, including the Laboratory Temperament Assessment Battery[14] and human intruder tests. These tests have been used extensively in studies of socioemotional development in macaque monkeys[15] and therefore are well-validated and highly translational tools. Indeed, the use of these paradigms is a significant strength of studies in nonhuman primates where tests can be given in similar formats to humans and monkeys. As another example, the ongoing Mayo Anesthesia Safety in Kids study[16] includes an operant behavioral testing battery that has been validated for developmental research in both humans and monkeys.[17]

Coleman *et al.*[10] found abnormal motor reflexes at 1 month of age (about 3 weeks after anesthesia exposure), as well as heightened anxiety in the home-cage social environment at 12 months of age in monkeys that were exposed three times to isoflurane (for 5 h each time) between postnatal days 6 and 12. Changes in these measures were not statistically significant in monkeys exposed to isoflurane only once for 5 h on postnatal day 6. Because monkeys did not undergo surgery, changes in anxiety in monkeys cannot

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be attributed to the experience of postoperative pain. These findings suggest that negative behavioral changes in children after surgery with general anesthesia[18] might result, in part, from long-term effects of multiple exposures to the anesthetic agent during the surgical procedure.

Because of the time and expense involved in a prospective study with nonhuman primates, investigators have based their anesthesia protocols on durations of exposure that are known to result in increased neuro- and glioapoptosis,[12,17,19,20] a candidate mechanism for the long-term effects of pediatric anesthesia on behavior. Because these durations tend to be longer than those commonly encountered in the pediatric operating room, many of these studies face the criticism that the anesthesia exposures are not clinically relevant. Single anesthesia exposures in infants that have been investigated recently[8,9] are of short duration relative to those in the studies to date with monkeys. It is worth noting that prolonged anesthesia exposure is certainly not unknown in pediatric surgery; Coleman *et al.*[10] cite 30% of infant anesthesia exposures being longer than 3 h at their institution, and prolonged sedation in the neonatal intensive care unit may last for weeks.[17] However, repeated exposures to anesthesia appear rare in these populations; for example, 7.4% of cases (44/593) in the study by Wilder *et al.*[4] received three or more anesthetic exposures, so three long exposures to anesthesia, especially within a week, would be extremely unusual in a clinical context. Although this is a limitation of extant preclinical studies, these exposure protocols establish boundary conditions upon which future work can build, to carry out more finely grained analyses of duration and frequency of anesthesia exposure.

Another issue in preclinical studies, especially with nonhuman primates, is that of repeated testing with a relatively small subject pool. This is a practical constraint of research with rare and expensive animals. Although this can incur statistical concerns about a large number of tests on a limited population, it is also a strength of these studies that data can be collected on the same study population longitudinally and across a number of different behavioral domains. As multiple research groups carry out studies using similar behavioral protocols, the generality of individual findings becomes clearer, as in all other areas of biomedical research. Similarly, this study like others tests both male and female monkeys but is underpowered to detect subtle sex differences. Nevertheless, this is a more desirable state of affairs than limiting preclinical studies to a single sex to evade this criticism.[21]

Two studies in monkeys[10,22] have independently reported increased anxiety-related behaviors in monkeys that were repeatedly exposed to general anesthesia as infants despite using different anesthetic agents and different schedules and durations of exposure. The finding that repeated exposure is associated with adverse neurocognitive outcomes is consistent with some human epidemiologic studies.[4,23] Recent prospective studies in humans have emphasized the safety of single, relatively brief exposures to general anesthesia in the context of pediatric surgery[8,9]; Coleman *et al.*[10] also find limited effects of single exposures to general anesthesia in monkeys. These observations reinforce one another and suggest that concerns about repeated or prolonged exposure of children to general anesthesia remain significant. Long-term neurobehavioral changes after repeated exposure to anesthesia in infancy may not simply be a consequence of cumulative exposure to the anesthetic agent; one study in rodents found a greater decline in synaptic density in adult rats exposed to sevoflurane three times for 2 h each as infants compared to those exposed once for 6 h,[24] so the mechanisms of long-term effects of repeated anesthesia exposure also merit further investigation.

The translation of these findings to the clinical setting remains subject to interpretation. It appears relatively easier to detect neurocognitive impairments in animal models after pediatric anesthesia than it does in clinical studies with humans. This may reflect uncertainty about the animal models that are used, with regard to details of anesthesia exposure, dosing, and so forth. It may also reflect the greater degree of experimental control in animal studies where all subjects receive identical anesthetic regimens, and behavioral assessments may be more extensive and sensitive to subtle effects.

Findings that single exposures to general anesthesia for pediatric surgery are not associated with adverse neurocognitive outcomes[8,9] are a great relief to anxious parents (and anesthesiologists). However, it does not seem safe to conclude that because single exposures to general anesthesia for pediatric surgery are apparently without substantial long-term adverse neurocognitive effects, any pattern of pediatric anesthesia exposure will be similarly benign. This work with nonhuman primates forms a critical middle ground for future investigations of impaired neurobehavioral function after repeated and/or prolonged anesthesia exposure and for testing potential interventions to mitigate them.

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Competing Interests

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