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Authors

Herrington, Joshua Anthony
Del Rosso, Laura
Capitanio, John P

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Behavioral effects of postnatal ketamine exposure in rhesus macaque infants are dependent on MAOA-LPR genotype

Joshua Anthony Herrington[1], **Laura Del Rosso**[2], **John P. Capitanio**[2]

[1]Department of Psychology, University of California Davis, Davis, California

[2]California National Primate Research Center, University of California, Davis, California

Abstract

Ketamine is an N-methyl-D-aspartate (NMDA) receptor antagonist widely used in pediatric anesthetic and therapeutic practices and veterinary medicine. Previous evidence suggests that exposure to ketamine during sensitive periods of development results in neural apoptosis and atypical behavior. Since monoamine neurotransmitters play important roles in prenatal and early postnatal neural development, and since previous work suggests ketamine can inhibit monoamine transporters, we hypothesized that there would be behavioral consequences of prenatal and early postnatal exposure to ketamine moderated by genotype of the promoter in the monoamine oxidase-A (*MAOA*) gene. From a large sample of animals ($N = 408$), we compared groups of rhesus monkeys that had experienced a single exposure to ketamine during prenatal development, an exposure during prenatal development and one postnatal exposure, a postnatal exposure with no prenatal exposure, and no exposures. Animals were classified by putative activity levels for the *MAOA* genotype and were tested between 3 and 4 months of age on a battery of behavioral tests. Results suggested that animals exposed to ketamine postnatally, at a dose typically used for sedative effects that also had the low-activity variant of *MAOA* performed poorly on a visual memory test compared to animals with the high-activity variant of the *MAOA* gene.

1 | INTRODUCTION

Ketamine, an N-Methyl-D-aspartate (NMDA) receptor glutamatergic antagonist, is an anesthetic that has a wide range of experimental and clinical uses. Ketamine is a preferred anesthetic in pediatric medicine (Deasy & Babl, 2010) due to its high dissociative effects and minimal impact on the respiratory system. Every year, approximately 1.5 million children in the United States undergo medical procedures that require anesthesia (Kozak, DeFrances, & Hall, 2006), and ketamine has been described as “among the most widely used agents for procedural sedation and analgesia in pediatrics (Buck, 2016).” In 2014, it was estimated that one of every 10 infants (9.57%) was born preterm (Martin, Hamilton, Osterman, Driscoll, & Mathews, 2017), and while advances in pediatric medicine have led to life-saving interventions in preterm infants, concerns over the potential adverse effects of exposures to ketamine have necessitated the need for studies to address potentially

Correspondence: Joshua Anthony Herrington, Department of Psychology, University of California Davis, Davis, CA, USA. Jaherrington@ucdavis.edu.

deleterious effects on central nervous system development (Olney, Young, Wozniak, Jevtovic-Todorovic, & Ikonomidou, 2004).

Interest in the effects of ketamine and other forms of general anesthesia on neurodevelopment significantly increased after it was found that, in addition to acting as an NMDA receptor antagonist, ketamine can also act on opioid receptors and monoamine transporters, among others (Kohrs & Durieux, 1998; Williams et al., 2018). In addition, while ketamine's primary effect is on the glutamate system, such effects may be dose dependent and could also involve non-NMDA receptors. For example, a microdialysis study in rats found that sub-anesthetic doses of ketamine (10–30 mg/kg) increases extracellular glutamate in the prefrontal cortex, while anesthetic doses (at 50 mg/kg) have no effect or a small decrease (with a 200 mg/kg dose) in glutamate (Moghaddam, Adams, Verma, & Daly, 1997).

Nonhuman primates provide great value as a model to study the effects of prenatal and early postnatal ketamine exposure on brain development and behavior. Not only do nonhuman primate models allow for the adequate monitoring of cardiovascular parameters but also their developmental trajectory, physiology, metabolic capability, pharmacology, and reproductive systems more closely resemble those of humans compared to other animals (Dobbing & Sands, 1979). In human and nonhuman primates, the majority of neural development takes place during the prenatal and neonatal period. During prenatal development, ketamine crosses the placenta readily, with cord blood levels higher than the mother in about 1.5 min (Ellingson, Haram, Sagen, & Solheim, 1977). The mechanistic effects of ketamine on monoaminergic function during the prenatal and early postnatal periods in humans and nonhuman primates remain largely unknown; however, there is some evidence to support the role of ketamine in altering dopamine D2 and serotonin 5HT2 receptors in rodent in vitro studies (Kapur & Seeman, 2002; Seeman, Ko, & Tallerico, 2005). There is also evidence in rats that a 50 mg/kg intra-peritoneal injection of ketamine can elevate brain monoamine levels, specifically epinephrine, serotonin, and 5-hydroxy indoleacetic acid (Kari, Davidson, Kohl, & Kochhar, 1978). The role of NMDA receptor function and glutamate/GABA systems in neural development, especially during prenatal and early postnatal synapse formation and elimination, has been well established (Komuro & Rakic, 1993; Lujan, Shigemoto, & Lopez-Bendito, 2005; McDonald & Johnson, 1990; Monyer, Burnashev, Laurie, Sakmann, & Seeburg, 1994; Scheetz & Constantine-Patton, 1994; Wenzel, Fritschy, Mohler, & Benke, 1997). Antagonizing NMDA receptors during prenatal development can lead to significant neural apoptosis in the perinatal brain (Lipton & Nakanishi, 1999). In fact, studies in nonhuman primates have shown that ketamine exposure at anesthetic doses during the third trimester (days 120–123 of a 165-day gestation) or in the early postnatal period (postnatal days 5–6) can lead to both necrotic and apoptotic neuronal cell death (Slikker et al., 2007).

Since ketamine can influence monoamine levels in the brain, monoamine genotype could act as a potential mediator for the effects of ketamine exposure during prenatal and early postnatal development. One of many genes that could regulate monoamine neurotransmission in the brain is monoamine oxidase-A (*MAOA*), an X-linked gene. MAOA breaks down monoamine neurotransmitters enzymatically after their release from the

terminal of a synapse, before levels reach prerelease amounts in the synaptic cleft. Recent evidence in rodents (Mejia, Ervin, Baker, & Palmour, 2002) and rhesus macaques (Capitanio, Del Rosso, Calonder, Blozis, & Penedo, 2012) suggests a role for *MAOA* in brain development during the prenatal period. Individuals with the less efficient, low-transcriptional activity, genotypes have been historically associated with atypical psychosocial behaviors, especially in conjunction with early life adversity, including exposure to drugs (Karere et al., 2009; Wakschlag et al., 2010). A recent study in rhesus macaques found a *MAOA* genotype-dependent effect of exposure to repeated sub-anesthetic doses of ketamine during prenatal development (Capitanio et al., 2012). Rhesus macaques possess a parallel polymorphism to humans for *MAOA* in the promoter region of the gene (*MAOA-LPR*, Newman et al., 2005), involving seven copies of an 18 bp repeat that confers low transcriptional efficiency and five or six copies conferring high transcriptional activity. As such, rhesus macaques are an excellent animal model to study the effect of *MAOA* genotype on atypical behavioral outcomes (see Karere et al., 2009; Capitanio et al., 2012).

The aim of the current study was to examine the effects of a single prenatal or postnatal sedative ketamine exposure, or a sedative ketamine exposure in both the prenatal and postnatal period of development, on measures of recognition memory, response to novelty, and behavioral responsiveness, and to determine if the effects were mediated by *MAOA-LPR* genotype. Our study is a continuation of the Capitanio et al., 2012 study, which found that indoor-reared rhesus infants that had 6+ prenatal exposures to ketamine, at sedative doses, displayed behavioral abnormalities, but only in those monkeys that possessed the low-activity *MAOA-LPR* genes. In the present study, we examine a much larger sample of animals whose prenatal and postnatal experiences involved development in half-acre, outdoor corrals that provided a richer physical and social environment compared to the living situation of animals in the previous study (Capitanio et al., 2012). Moreover, whereas the animals in the earlier study received one to nine exposures to ketamine prenatally (and zero or one exposure postnatally), animals in the current sample had exposures ranging from 0 to 2 for the prenatal and early postnatal period combined, enabling us to make comparisons with animals that had no ketamine exposure at all. We note that the doses used in our previous and our current study are sedative doses (i.e., 10 mg/kg), and not the anesthetic doses (often involving intravenous infusion of up to 50 mg kg hr^{-1}) that have been used to examine the neural consequences of early exposure in other studies. Sedative doses are most commonly used in veterinary practice, but the long-term behavioral and neural consequences, particularly during sensitive periods of brain development, remain largely unknown.

Our primary hypothesis was that we would find behavioral differences similar to those reported by Capitanio et al. (2012) that were associated with prenatal and early postnatal exposure to sedative doses of ketamine and that were moderated by the low-activity *MAOA-LPR* gene: increased activity, poor performance in a memory task, reduced emotionality, and less contact with novel objects. The BioBehavioral Assessment (BBA), from which the current behavioral data are drawn, includes additional social and emotional tests, such as the human intruder test, which have been shown to be affected by repeated anesthetic doses in the first few months of life in previous studies of nonhuman primates (Coleman et al., 2017; Raper, Alvarado, Murphy, & Baxter, 2015; Raper, De Biasio, Murphy, Alvarado, & Baxter,

2018). For the current study, we chose to base our predictions on the tests that were found to be significantly affected by sedative doses of ketamine in low-activity *MAOA* genotype monkeys in the Capitanio et al., 2012 study. We further predicted that the effects of ketamine exposure in animals with the low-activity genotype would be muted, as animals in the current study were only exposed to 1–2 doses of sub-anesthetic ketamine and were raised in outdoor corrals in rich social and physical environments that we have found can mitigate the negative effects of the low-activity *MAOA-LPR* genotype (Karere et al., 2009).

2 | METHODS

2.1 | Subjects and housing

We studied behavior in infant rhesus macaques that were exposed to ketamine either prenatally and/or in the ~3 months following birth. Subjects were $N = 408$ (264 male) infant rhesus monkeys (*Macaca mulatta*) reared in half-acre outdoor corrals that were either Specific Pathogen Free (SPF) or conventional (i.e., non-SPF). The animals were a mean of 106.29 days of age ($SD = 10.08$) at the time of biobehavioral testing. Animals for the study were drawn from a larger pool of animals (1) if they were born at the CNPRC between the years 2011 and 2013; (2) if they had one prenatal, one postnatal, one prenatal and one postnatal, or no exposures to ketamine (see Table 1) that were only for routine physical examinations; (3) whose estimated conception dates met our criteria (see next paragraph); and (4) if they had participated in the CNPRC's BBA program at 3–4 months of age.

2.2 | Ketamine exposures

Ketamine was intramuscularly administered at a dose of 10 mg/kg for each animal by trained CNPRC animal care staff during semiannual physical health checks. We obtained cases for the current study over a period of 3 years, during which ketamine was purchased from several vendors under different brand names (vetaket, ketaset, keta-ject, and ketaved). All products consisted of racemic mixtures of ketamine HCl. We first classified animals exposed prenatally to ketamine by assuming a 165-day conception period (which is typical for this species), the subject's date of birth, and the date of ketamine exposure. To mitigate the uncertainty of trimester classification due to having only an estimated date of conception, we eliminated animals from our subject pool at the junction of two trimesters by creating the following classifications: first trimester (prenatal days 5–50), second trimester (prenatal days 60–105), and third trimester (days 115–165). For individuals that experienced a ketamine exposure postnatally ($n = 88$), the mean age was 76.23 days (range: 7 to 115 days). Table 1 indicates how the 408 subjects were distributed across prenatal exposure, postnatal exposure, and *MAOA* genotype.

2.3 | Behavioral assessments

The current retrospective study was conducted by analyzing data previously collected from the California National Primate Research Center's (CNPRC) BBA program (Capitanio, Mason, Mendoza, Del Rosso, & Roberts, 2006; Golub, Hogrefe, Widaman, & Capitanio, 2009). Mothers and infants were net captured from their field corrals, separated from each other, and delivered to the testing room (infants) or to holding cages (mothers) that were outside of olfactory, visual, and auditory range of the infants. Infants, which were always

tested in cohorts of 5–8 animals, arrived at 0900 hr, and were housed individually in standard-sized holding cages (0.58 × 0.66 × 0.81 m, Lab Products, Maywood, NJ) indoors. Infants were returned to their mothers at 1000 hr the following day, where they were allowed an hour to nurse prior to return to their natal cages with their mothers. Each holding cage contained a stuffed cloth toy duck, a towel, and a novel object that the infants could manipulate. Infants were provided with water ad libitum, orange-flavored drink, fresh fruit, and commercial monkey chow.

The BBA program comprises a series of biobehavioral tests over a 25 hr period that assesses a variety of behavioral and physiological characteristics. Some tests were conducted within the infants' holding cages, and some consisted of transferring the animals to a testing cage in an adjacent room. Behavior was coded using version 5 of The Observer software program (Noldus, 1991). Intra- and interobserver reliabilities for assessing behavior were checked regularly and exceeded 85% agreement. Data from the assessments below were used in the current study, inasmuch as these assessments demonstrated effects of ketamine exposure in our previous study (Capitanio et al., 2012).

2.3.1 | Preferential look—The preferential look test is a standardized assessment of visual recognition memory that requires no previous training and has been previously found to differentiate typical from atypical populations of monkeys (Bachevalier, Brickson, & Haggar, 1993; Golub, Slotkin, Tarantal, & Pinkerton, 2007; Gunderson, Grant-Webster, Burbacher, & Mottet, 1988; Gunderson, Grant-Webster, & Fagan, 1987). The preferential look task that we implemented in the BBA program was described in Sclafani et al. (2016). In brief, at approximately 1130 hr, each subject was moved from its temporary holding cage to a test cage (0.39 × 0.41 × 0.46 m) that was stationed 0.69 m from a video screen (Panasonic KV32540, Secaucus, NJ, 78.7 cm diagonal screen) that was connected to a DVD player (Panasonic DMRT3040). A lowlight camcorder (KTL KPC-S20P, Northern Video, Rocklin, CA) was positioned in the top center of the viewing monitor to record the animals' responses. The video stimuli—pictures of unfamiliar monkeys—had been previously recorded, and comprised seven problems, each of which contained: 5 s of blank screen, 20 s familiarization trial where a single picture was presented as two identical side-by-side images separated by 25.4 cm of white space, 5 s blank screen, 8 s test trial in which the now-familiar image was paired with a novel picture located on either the left or right side (the order of novel stimuli location was randomized across the seven problems), 5 s blank screen, and a second 8 s test trial (the same two images as the previous test trial, with stimuli presented on opposite sides of the screen). A 100-Hz tone was presented 250 milliseconds prior to test trials in order to orient the animal's gaze. All video stimuli were photographs of novel rhesus macaque monkeys and can be found as Supplementary Material to Madrid et al. (2017). The principal outcome measure was the proportion of looking time spent viewing the novel pictures, which was calculated by taking the duration that the animals looked at the novel stimuli for the two test trials for each problem, and dividing by the duration of time animals looked at either the novel or familiar stimuli. A mean was taken across the seven problems. During the preferential look test, the animals (which were unrestrained) were free to look at the stimuli or not, so we scored a "balk" for a problem if the animal did not look at the stimuli during the familiarization trial, or if the animal did not look at either the novel or

familiar stimuli during the test trials of a problem. The number of balks was a second outcome measure.

2.3.2 | Novel object interaction—Each temporary holding cage contained a novel object: a black PVC cylinder (3.8 cm diameter × 8.9 cm long weighing 0.09 kg) that was hollow and contained an actigraph device (Actiwatch, Philips Respironics, Andover, MA) that recorded whenever an animal exerted force on the object. We used the factory default setting for the sensation/activation threshold and found that the Actiwatches are activated only when the infants manipulated the object directly, and in the very rare occurrence of a vigorous cage shake. The novel object was removed at approximately 1615 hr. The data collected from the actigraphs were separated into 5 min blocks of time, and within each block, the number of 15 s intervals during which the animal exerted force on the object was calculated. We discarded any data for when either the animal or the object was placed in, or removed from, the holding cage. Data were summarized as the mean number of 15 s intervals of contact per 5 min block, with a maximum score of 20, for two time periods. Time period 1 consisted of the initial entry of the novel object into the cage at approximately 0900 hr until approximately 1230 hr. Time period 2 was from 12:30 hr until the removal of the novel object at 1615 hr.

2.3.3 | Behavioral responsiveness—Each animal was observed by a technician for two 5 min periods: once approximately 15 min after relocation to the holding cage (0915 hr) on Day 1 of the BBA and again at 0700 on Day 2. An observer sat approximately 2.4 m in front of the holding cage while avoiding eye contact with the subject. All behaviors were scored according to the ethogram in Golub et al. (2009). Frequencies of behaviors were converted to a rate per 60 s interval, and durations were converted into a proportion of total time observed score. Data from the larger BBA program (>1,400 animals) were treated to exploratory and confirmatory factor analyses (Golub et al., 2009), and two factors were identified: “Emotionality” comprised the rate of vocalizations [barks and coos]; and whether the subject displayed lipsmacks, threats, and self-scratching behaviors; and “Activity” that comprised the proportion of time locomoting; proportion of time an animal was not in a hanging position on the front, sides, or top of the cage; rate of environmental exploration; and whether or not the animals drank, ate food, or were in a crouched posture. The Day 1 and Day 2 data were summarized by z-scoring each item, then creating the factor analysis-derived scales, which were then re-z-scored to have a mean of zero and $SD = 1$. Day 1 z-scored values generally reflect responsiveness to maternal separation and relocation, and Day 2 values are a general reflection of the animal’s adaptation to the BBA testing procedure.

2.4 | Genotyping

Methods for genotyping have been described in Karere, Sullivan, Kinnally, Capitanio, and Lyons (2012); the same methods were used in the present study, except that the Taq polymerase was purchased from a different vendor (Promega, Madison, WI). Briefly, *MAOA* alleles were classified according to repeat number of 5 (240 base pairs), 6 (258 bp), and 7 (276 bp). As the *MAOA* gene resides on the X chromosome, animals were classified as “low-activity” ($n = 145$) for *MAOA* genotype if they were 7/7 homozygous (female) or

7/− hemizygous (male) and were classified as “high-activity” (n = 263) for 5/5 (or 5/−), 6/6 (or 6/−), or 5/6 genotypes (Newman et al., 2005).

2.5 | Statistical analysis

To test the hypothesis described above, we created 4 × 2 × 2 general linear models (GLM) of trimester (no prenatal exposure, exposure in the 1st, 2nd, or 3rd trimester) × *MAOA* genotype (low activity vs. high activity as described above) × postnatal exposure (one postnatal exposure vs. no postnatal exposure) with post hoc Bonferroni-adjusted pairwise comparisons for the outcome variables in the preferential look, novel object, and behavioral responsiveness tests. Our significance threshold was set at $p < 0.05$ and effect sizes are reported as partial eta squared. Additional covariates included cohort year, SPF status (yes or no), and sex. For the activity and emotionality variables in the behavioral responsiveness test, a four-way ANOVA for the Day 1/Day 2 activity measures with day as a repeated measure was used. Below, we report all results for those variables showing main or interaction effects for ketamine exposure.

3 | RESULTS

The impact of postnatal ketamine exposure was greatest for animals with the low-activity *MAOA* genotype for performance in the preferential look task, as indicated by a significant three-way interaction of trimester × *MAOA* type × postnatal exposure; $F(3, 389) = 2.787$; $p = 0.041$, $\eta_p^2 = 0.021$ (Figure 1a–d). Follow-up analyses for each prenatal exposure group (no exposure prenatally, trimester 1, trimester 2, or trimester 3 exposures) found a two-way interaction between *MAOA* classification (high vs. low) and postnatal exposure (yes vs. no) only in animals that had not been prenatally exposed to ketamine (see Figure 1a; $F(1, 175) = 5.13$; $p = 0.025$, $\eta_p^2 = 0.030$). Linearly independent pairwise comparisons among the estimated marginal means of postnatal exposure (yes vs. no) for low- versus high-activity variants of the *MAOA* gene found a near-significant ($F(1, 166) = 3.77$; p=0.054) effect of postnatal ketamine exposure (Figure 1a): among animals with the low-activity variant of *MAOA*, animals that were exposed to ketamine within the first 3 months after birth had worse performance than animals that did not have a postnatal exposure; no significant effects were found for balks in the preferential look task.

For the full sample (n = 408), mean proportion of time spent looking at the novel stimuli was 0.545 (95% CI: 0.533–0.558), a number that compares favorably with the values for the full sample from the BBA program (mean: 0.546, 95% CI: 0.542–0.550, n = 4,469). Inspection of means and confidence intervals for the 16 groups of animals in the present study revealed that performance was best among animals that got no prenatal exposure to ketamine (Figure 1a): three of the four groups (defined by MAOA status and postnatal exposure status) had mean values that were significantly greater than chance (i.e., the 95% CI did not contain 0.50); the group that did not perform better than chance was the group with the low-activity MAOA genotype that received a postnatal exposure, as described above. Among the animals with a prenatal exposure during the first trimester (Figure 1b), none of the four groups had mean values that exceeded chance responding. For those animals that received a prenatal exposure in the second trimester, only one group showed responding at greater than chance

levels (animals with the high-activity genotype and with a postnatal exposure; Figure 1c, second bar). Finally, among animals with a prenatal exposure in the third trimester, two groups showed responding at greater than chance levels; animals with the high-activity genotype and no postnatal exposures, and animals with the low-activity genotype that did receive a postnatal exposure (though we note that there were only four animals in this cell) (Figure 1d, first and fourth bars).

For the Day 1/Day 2 activity measures in the behavioral responsiveness test, a main effect of trimester $F(3, 408) = 3.40$; $p = 0.018$, $\eta_p^2 = 0.026$ was found; animals exposed to ketamine in the second trimester were significantly more active compared to animals not exposed prenatally to ketamine ($p = 0.028$) and to animals exposed to ketamine in the third trimester ($p = 0.026$) (see Figure 2). We also found a significant main effect of *MAOA* classification $F(1, 408) = 3.90$; $p = 0.049$, $\eta_p^2 = 0.010$; animals with the low-activity genotype displayed significantly higher activity compared to animals with the normal-activity genotype. No significant effects involving ketamine exposure were found for novel object interaction (Figure 3) or for emotionality (see Table 1).

4 | DISCUSSION

Our study demonstrated an influence of postnatal ketamine exposure at sedative doses on visual recognition memory in 3- to 4-month-old rhesus macaques. The effect was moderated by *MAOA* genotype in that, among animals that received no prenatal exposure to ketamine, performance among individuals with the low-activity *MAOA* genotype (7/7 for females and 7/− for hemizygous males) was impaired compared to animals with a similar genotype that were not exposed postnatally. Moreover, the low-activity genotype animals with a postnatal exposure were the only group of animals, among those with no prenatal exposures, whose performance was at chance levels on this test. In addition, there was evidence that any prenatal exposure to ketamine may be problematic: animals that received a single prenatal exposure to ketamine in the first trimester (Figure 1b), and those in three of the four groups that received a single exposure in the second trimester (Figure 1c), all responded at chance levels; animals that received a prenatal exposure in the third trimester (Figure 1d) performed adequately on this task, but only if they received no postnatal exposure and had the high-activity genotype (we discount the result of the other group in this condition, owing to small sample size). Finally, for our measure of activity in the behavioral responsiveness test, we found no moderation effect of genotype, which was our second principal result; rather, the animals exposed to a single dose of ketamine in the second trimester were more active in their holding cages on both days of observation compared to animals exposed in the third trimester, or those with no prenatal exposure to ketamine, regardless of *MAOA* genotype or postnatal exposure status. No prenatal effects were observed for any of the other behavioral tests (novel object contact and emotionality). Below, we discuss the implications of our findings in light of a previous study in this series (Capitanio et al., 2012), and consider the impact of our study compared to studies by others (largely conducted with anesthetic, not sedative, doses) suggesting that ketamine has a deleterious effect on behavior, particularly for individuals with the low-activity *MAOA* genotype.

Several studies in a variety of mammalian species have found that administration of NMDA receptor antagonists like ketamine during the late prenatal or early postnatal period results in cognitive deficits, such as learning and memory impairment (Fredriksson, Archer, Alm, Gordh, & Eriksson, 2004; Mickley, Remmers-Roeber, Crouse, & Peluso, 2000; Mickley et al., 1998). A previous study in rhesus macaques that were tested through late adolescence found that 24 hr of ketamine anesthesia on postnatal days 5 or 6 resulted in problems with a repeated acquisition task, which measures novel sequence learning (Paule, Lazar, Akinkuotu, Adesina, & Olutoye, 2015). In contrast, the principal results from the Capitanio et al., 2012 study, which involved sedative doses, were mainly for affective responses in rhesus macaques that were repeatedly exposed to ketamine during prenatal development and possessed the low-activity *MAOA* genotype. Specifically, Capitanio et al., 2012 found that multiple exposures to ketamine in the first trimester (the range of exposures was 1–7) in animals that had the low-activity *MAOA* genotype expressed a pattern of behaviors that suggested general behavioral inhibition, with reduced emotionality and reduced contact with novel objects. In comparison, the present study found no evidence to suggest that a single prenatal and/or postnatal dose of ketamine can affect indicators of behavioral inhibition, emotionality, and novel object contact. In an interesting contrast to the Capitanio et al., 2012 study, we found no evidence that balking behavior (which is probably associated with elevated affect) was affected during the preferential look task; rather, we found evidence that performance in the preferential look task was significantly lower in animals that had been exposed to one dose of ketamine in the prenatal and early postnatal period. Our current results provide further evidence that at least some consequences of ketamine exposure are dependent on *MAOA* genotype in rhesus macaques and suggest that the preferential look task, which measures visual recognition memory, might be a sensitive indicator of cognitive status.

We had anticipated that the effects of ketamine exposure in the current study may have also been mitigated by the fact that our animals were reared in outdoor field corrals. Animals that are reared in the CNPRC's half-acre outdoor enclosures experience complex social interactions and other forms of regular environmental enrichment. Behavioral interventions like environmental enrichment have been previously shown to attenuate the neurobehavioral impairments induced by general anesthesia in rats (Shih et al., 2012). Animals in the Capitanio et al., 2012 study were reared indoors and lacked the same socially and environmentally dynamic setting as the outdoor-reared animals in the present study; in fact, an earlier study examining early rearing history (Karere et al., 2009) found behavioral differences depending on *MAOA* genotype in monkeys reared in the nursery, indoors with their mother (identical to the rearing situation in the Capitanio et al. [2012] study), and in small outdoor groups, but not in monkeys reared in the large outdoor corrals, as in this study. In fact, the effect sizes for the current study were small relative to those seen in our earlier report. We note that studies in other laboratories have found behavioral deficits in rhesus macaques reared in complex social groups similar to ours, but these animals were exposed to multiple anesthetic doses in the first few months after birth (Coleman et al., 2017; Raper et al., 2015, 2018). As such, the present results suggest that the rich environment of the field corral setting might buffer an individual but only up to a point, for example, involving only a single, sedative dose of ketamine postnatally. Future studies should address whether multiple

exposures of sub-anesthetic doses or higher anesthetic doses of ketamine in monkeys with the low-activity *MAOA* genotype raised in a complex social environment has a more pronounced effect on behavior that we saw in the current study.

Regardless of the species or animal model used, general anesthetics like ketamine administered during sensitive periods of development at anesthetic and sub-anesthetic doses have deleterious effects on the developing brain (Brambrink et al., 2012; Creeley et al., 2014; Olutoye et al., 2015; Rizzi, Carter, Ori, & Jevtovic-Todorovic, 2008; Rizzi, Ori, & Jevtovic-Todorovic, 2010; Slikker et al., 2007; Walters & Paule, 2017). The exact period of development during which a rhesus macaque's brain is sensitive to the effects of ketamine is still unclear (Schenning et al., 2017). For rodents, it is apparent that this period spans the first postnatal weeks. In monkeys, some evidence has shown the period of sensitivity to neuronal cell death induced by general anesthesia, including ketamine, extends to postnatal day (PND) 7 (Walters & Paule, 2017), while another study suggests that the developing brain is sensitive to isoflurane up to postnatal day 40 (Schenning et al., 2017). We found that performance on the preferential looking task was impaired in most animals that received a single sedative dose prenatally, though we acknowledge that some cell sizes were small. Moreover, results from the group that experienced no prenatal exposure suggest that the period of postnatal sensitivity might be broader than previously identified, at least for animals possessing a "risky" *MAOA* genotype. Although our findings might extend the sensitive period of time that ketamine could potentially alter behavior in rhesus monkeys, a future prospective study is needed to uncover the specific mechanism by which an animal with the low-activity *MAOA* gene exposed to a single sub-anesthetic dose of ketamine could experience maladaptive effects on behavior.

We acknowledge that our results, demonstrating behavioral consequences of single sedative doses of ketamine delivered postnatally, might be considered at variance with published reports demonstrating that larger, anesthetic doses, delivered at 5 days of age postnatally for 3 hours, showed little neuronal cell death (Slikker et al., 2007). As described below, more research involving precise timing of administration, as well as mechanistic studies, will be needed to fully address these discrepancies. It is important to note, however, that the studies examining behavioral and neural consequences (or their absence) of anesthetic doses of ketamine typically involve very small sample sizes (e.g., in the Slikker et al., 2007 studies, each cell contained $n = 3$), which would only find extremely large effects of the dosing. While we also have a few cells with single digit subject numbers, overall, our study, which involves $n = 408$ animals, is powered to find much smaller effects, as we report above. Our large sample size, combined with our hypothesis-driven analytical approach that was based on prior work (Capitanio et al., 2012), provides some confidence that our results, although of small effect, show real consequences for monkeys receiving sedative doses of ketamine.

4.1 | Limitations and conclusion

We recognize several limitations in the present study. First, ours was a retrospective study that utilized healthy pregnant female macaques that had only received ketamine for sedation during semiannual health checks and at no prespecified points in gestation. Controlled timing of prenatal ketamine exposures done in a prospective way would be the preferred

method of further examining the role of sedative doses of ketamine in development. Second, since all of our subjects were conceived in outdoor corrals, we did not know the exact date of conception; as described in our Methods, however, we attempted to sharpen the trimester contrasts by excluding animals from our study if their estimated conception dates were within ±5 days of the adjacent trimester. A prospective study with known dates of conception and known dates of exposure to ketamine could further illuminate sensitive periods of prenatal exposure to ketamine in nonhuman primates. Future prospective studies might try to evaluate the number of prenatal exposures or determine if higher amounts of ketamine (i.e., anesthetic plane doses of 3+ hours) could have a broader effect on animal behavior, as we found only limited evidence (for activity) for the effects of a single prenatal dose of ketamine on postnatal behavior. In addition, because others have not found any effects of postnatal ketamine exposure on apoptosis and behavior (Slikker et al., 2007), it would be interesting to see if some effect might be evident for animals with the low *MAOA* variant. We also recognize that some of the cells in our study (Table 1) had fewer than 10 animals, which was why we did not conduct a detailed discussion of trends found in our data; while small cell sizes create power issues, we note that the effect that we report for performance on the preferential look test did involve reasonable numbers—$n = 45$ animals with no prenatal or postnatal exposures and with the low transcriptional variant of *MAOA* versus $n = 15$ animals with no prenatal exposure, a single postnatal exposure and with the low transcriptional variant of *MAOA*. Again, a prospective study could better balance the numbers in each condition.

As a final point, the present study does not illuminate the mechanism by which ketamine exposure in the sedative range results in reduced performance in a visual recognition task. At this point, the work that has been done examining the effects of anesthetic doses of ketamine provides the best guess as to what the mechanisms might be. We believe, however, that the large number of animals used in the current study, and the use of animals reared in a complex and enriched environment, gives us increased confidence that there is an effect, albeit a small one, of early postnatal exposure to ketamine that is mediated by *MAOA* genotype on measures of visual recognition memory. Our results contribute to a growing literature that suggests animal researchers, caretakers, and veterinary and medical doctors should consider that *MAOA* genotype could be an important variable in determining whether ketamine or other anesthetics might have long-term impacts on behavior across the life span.

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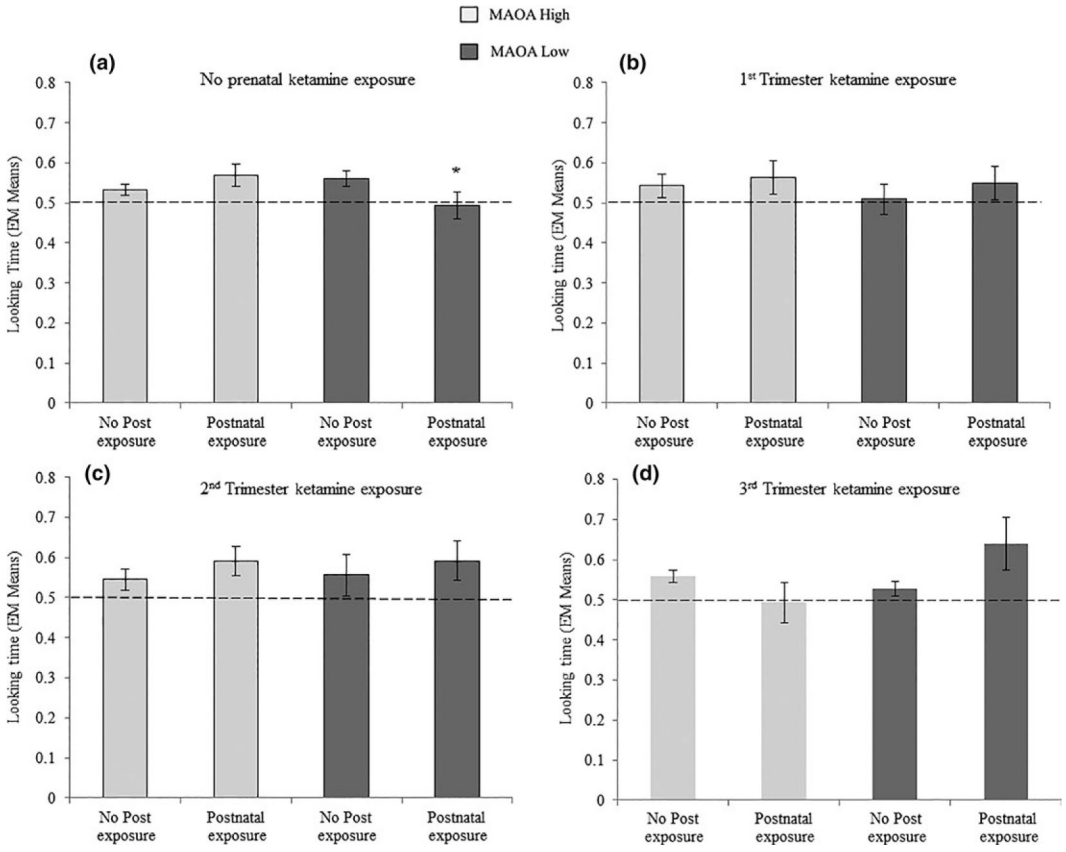

FIGURE 1.

A-1D: Estimated marginal means and standard errors for proportion of time spent gazing at a novel stimulus in a visual recognition memory test. The black dotted line indicates chance performance on this task. *$p = 0.054$

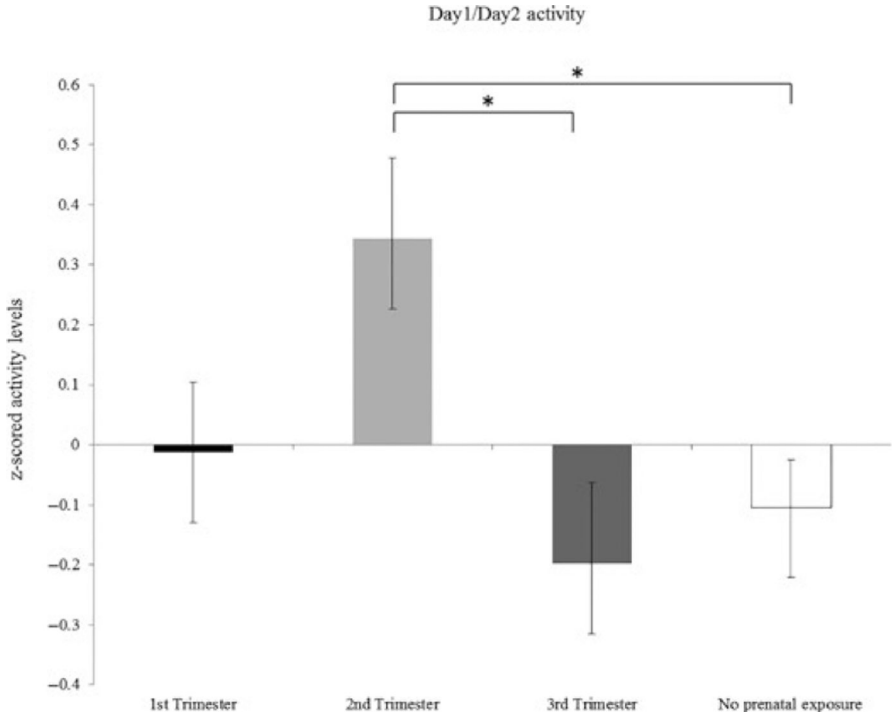

FIGURE 2.

Estimated marginal means and standard error for averaged *z*-scored Day 1/Day 2 activity levels for the behavioral responsiveness test.*$p < 0.050$

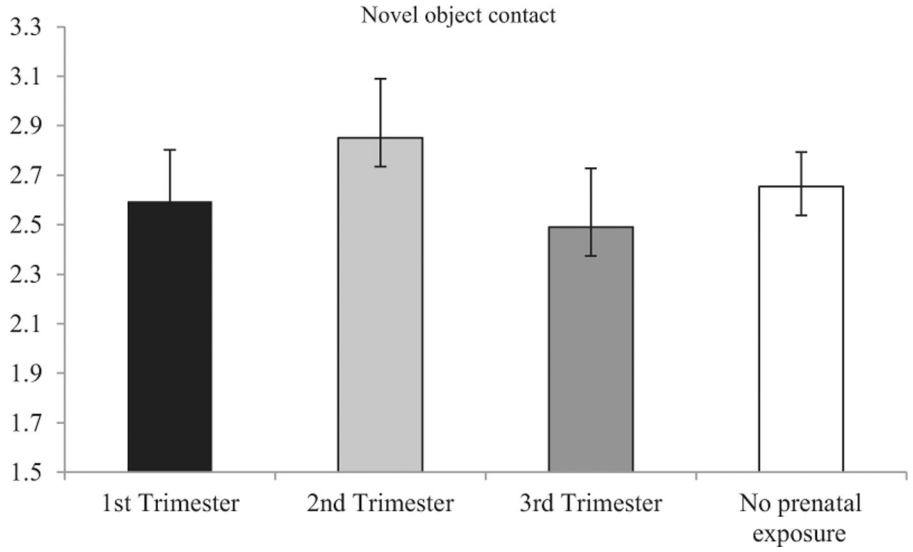

FIGURE 3.
Estimated marginal means and standard error for novel object contact averaged over both time periods

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TABLE 1

Group demographics and behavioral responsiveness

No prenatal exposure	***n***	***MAOA* low**	**Postnatal exposure**	**Day 1 activity *M* (*SD*)**	**Day 2 activity *M* (*SD*)**	**Day 1 emotionality *M* (*SD*)**	**Day 2 emotionality *M* (*SD*)**
	93	No	No	−0.05 (1.07)	−0.03 (1.09)	−0.12 (0.83)	−0.09 (0.94)
	22	No	Yes	−0.06 (0.85)	−0.09 (1.02)	0.18 (1.18)	−0.15 (0.70)
	45	Yes	No	−0.27(0.93)	0.10 (0.94)	0.25 (1.17)	0.05 (1.07)
	15	Yes	Yes	−0.21 (1.10)	0.06 (0.76)	0.39 (1.30)	0.21 (1.04)
Prenatal exposure (trimester)	***n***	***MAOA* low**	**Postnatal exposure**				
1	21	No	No	0.10 (1.10)	0.16 (1.13)	0.03 (0.92)	−0.44 (0.55)
1	10	No	Yes	−0.30(1.06)	−0.96 (0.82)	−0.14 (1.11)	−0.50 (0.50)
1	12	Yes	No	0.09 (1.30)	0.31 (1.0)	−0.06 (0.48)	0.07 (0.61)
1	10	Yes	Yes	0.09 (1.09)	0.37 (0.93)	−0.43 (0.45)	0.21 (0.79)
Prenatal exposure (trimester)	***n***	***MAOA* low**	**Postnatal exposure**				
2	26	No	No	−0.01 (1.09)	−0.03 (0.74)	−0.10 (1.03)	−0.29 (0.83)
2	13	No	Yes	0.52 (0.86)	−0.28 (1.10)	0.02 (1.21)	0.27 (1.57)
2	6	Yes	No	0.28 (1.08)	−0.01 (0.89)	0.37 (1.35)	1.40 (2.02)
2	7	Yes	Yes	0.89 (0.85)	0.93 (1.20)	0.16 (1.05)	0.17 (1.00)
Prenatal exposure (trimester)	***n***	***MAOA* low**	**Postnatal exposure**				
3	71	No	No	−0.09 (0.99)	−0.04 (0.86)	0.17 (1.28)	0.18 (1.16)
3	7	No	Yes	−0.27 (1.13)	−0.53 (1.43)	−0.49 (0.33)	−0.28 (0.67)
3	46	Yes	No	−0.11 (0.89)	−0.11 (0.95)	−0.20 (0.81)	−0.05 (0.97)
3	4	Yes	Yes	−0.11 (1.02)	−0.45 (0.77)	−0.49 (0.30)	0.59 (1.61)