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Behavioral effects of prenatal ketamine exposure in rhesus macaques are dependent on *MAOA* genotype

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Abstract

Ketamine is an NMDA receptor antagonist that is used in anesthetic, abuse, and therapeutic contexts. Recent evidence suggests that ketamine may affect not only glutamate systems, but may also act on receptors in the dopamine and serotonin systems. Because monoamine neurotransmitters play important trophic roles in prenatal development, we hypothesized that the behavioral consequences of prenatal exposure to ketamine may be moderated by genotype of the promoter in the monoamine oxidase-A (*MAOA*) gene. Eighty-two infant rhesus monkeys were identified that had known dates of conception and exposures to ketamine during gestation. Animals were tested at 3–4 months of age on a battery of tests assessing responsiveness to maternal separation, recognition memory, and contact with novel objects. Animals were classified by putative activity levels for the *MAOA* genotype. The effects of prenatal ketamine exposure were seen only in the context of *MAOA* genotype. Greater exposure to ketamine resulted in increased activity, less willingness to perform in the memory task, and reduced emotionality and novel object contact, but only for individuals with the low-activity genotype. Nearly all effects of exposure to ketamine were found in the first and second trimesters. *MAOA* genotype moderates the role of prenatal ketamine exposure at time points in gestation earlier than have been shown in past research, and are particularly evident for measures of emotionality. These results support the idea that the ketamine's use might be best considered in light of individuals' genetic characteristics.

Keywords

ketamine; *MAOA*; temperament; anxiety; rhesus monkey

Introduction

Ketamine is an N-methyl-D-aspartate (NMDA) receptor antagonist that has a variety of uses. It is used as an anesthetic in both pediatric (Deasy & Babl, 2010) and veterinary (Muir, 2010) practice, and it is a highly abused drug sought out for hallucinogenic and out-of-body experiences (Reynaud-Maurupt, Bello, Akoka & Toufik, 2007). Ketamine has also proven

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useful in understanding psychiatric disorders. For example, ketamine has been used in creating animal models of schizophrenia (Bubenikova-Valesova, Horacek, Vrajova & Hoschl, 2008), and most recently ketamine has been examined for its potential antidepressant effects (Machado-Vieira, Salvadore, Diazgranados & Zarate, 2009). These latter findings have provided hope for new psychiatric treatments through targeting of the glutamate system (Krystal et al., 2010).

Because ketamine is an NMDA antagonist, its principal effects are in the glutamate system, though ketamine's effects on glutamate appear to be dose-dependent and to involve non-NMDA receptors as well. In rats, for example, microdialysis studies have shown that subanesthetic doses (10–30 mg/kg) increase extracellular levels of glutamate in prefrontal cortex, while anesthetic doses produce either no effect (50 mg/kg) or a decrease in glutamate (200 mg/kg) (Moghaddam, Adams, Verma, Daly, 1997). Ketamine also has effects on other neurotransmitter systems, however, particularly the monoamines (Gunduz-Bruce, 2009). Early research in rats demonstrated that after a single ketamine injection, brain levels of epinephrine, serotonin, dopamine, and norepinephrine were significantly altered (Kari, Davidson, Kohl & Kochhar, 1978; Ylitalo, Saarnivaara & Ahtee, 1976). More recently, Moghaddam et al. (1997) showed that, in rats, a subanesthetic dose (30 mg/kg) elevated dopamine in the prefrontal cortex, presumably through non-NMDA receptors. While the exact mechanisms by which ketamine influences monoaminergic function remain unknown, there is some evidence suggesting that ketamine also exerts direct effects at dopamine D2 and serotonin 5-HT2 receptors (Kapur & Seeman, 2002; Seeman, Ko & Talerico, 2005).

The fact that ketamine has effects on a variety of neurotransmitter systems suggests that ketamine's behavioral and pharmacological effects might be greatest during the process of neural development, the majority of which takes place prenatally in primates, both human and nonhuman. Ketamine readily crosses the placenta, with cord blood levels greater than those of the mother in about 1.5 minutes (Ellingson et al., 1977). The role of NMDA receptor function in neural development (particularly in synapse formation and elimination) has been long-known (Scheetz & Constantine-Patton, 1994), and of the various ionotropic glutamate receptors, NMDA receptors appear earliest in prenatal development, with their antagonism resulting in considerable apoptosis (Herlenius & Lagercrantz, 2004). Studies with nonhuman primates have shown that ketamine exposure in the third trimester (days 120–123 of a 165-day gestation) or in the early postnatal period (postnatal days 5–6) can lead to neuronal cell death (Slikker et al., 2007) and can have long-lasting cognitive, and possibly motivational, consequences (Paule et al., 2011). These effects were not seen with exposure at postnatal days 35–37, however, a result consistent with the idea that much of the rhesus monkey brain growth spurt occurs prenatally and in the early postnatal stage (Dobbing and Sands, 1979; Granger et al., 1995). (We note that studies showing cognitive and apoptotic effects in monkeys utilized 24-hr anesthetic doses of ketamine; however, apoptosis has been seen in mice administered a single dose as low as 5 mg/kg neonatally: Rudin et al., 2005.)

While studies examining the effects of ketamine exposure on brain development have focused on the role that the NMDA receptor plays during the brain growth spurt period (late prenatal, early postnatal) in rodents and monkeys, it's possible that ketamine could exert behavioral effects earlier, including through its effects on early-developing brain monoamine systems. Autoradiograph studies show that monoamine neurons' mitosis is relatively complete by approximately the end of the first month of gestation in rhesus monkeys, which is about a month prior to the peak of neurogenesis in the target structures of these neurons (e.g., striatum and prefrontal cortex) (Levitt & Rakic, 1982). Neurotransmitters at these early time points are playing important trophic roles in the

prenatal brain and are contributing to brain organization (Nguyen et al., 2001). Disruption of this process could result in behavioral consequences.

Because ketamine can influence levels of monoamines, it's possible that ketamine's prenatal influence on brain and behavior could be moderated by an individual's monoamine-related genotype. There is a growing number of polymorphic genes relating to monoamine neurotransmission that have been identified (Sabol, Hu & Hamer, 1998), but one that has well-known neuropsychiatric associations is monoamine oxidase-A (*MAOA*), which is an X-linked gene. *MAOA* is a mitochondrial-bound enzyme that breaks down monoamine neurotransmitters following synaptic release and subsequent return of the neurotransmitters to the presynaptic terminal. Recent evidence (Cheng et al., 2010; see below) suggests a role for *MAOA* in prenatal brain development. A polymorphism in the promoter region (*MAOA-untranslated variable nucleotide tandem repeat*) includes variants that promote high or low transcriptional activity of the *MAOA* gene (D'Souza & Craig, 2006). Individuals with the less efficient, low-activity, genotypes (in humans, this comprises individuals with 3 or 5 copies of the 30-bp repeats; individuals with 3.5 or 4 copies are considered as possessing high-activity alleles) have generally been considered at-risk for psychosocial problems, particularly when those individuals have also experienced early adversity of some type (Sabol et al., 1998). Recently, the notion of an "adverse environment" has been reconsidered (e.g., Karere et al., 2009) and has been expanded to include drugs; for example, a recent study demonstrated that prenatal exposure to cigarettes and conduct disorder was moderated by *MAOA* genotype (Wakschlag et al., 2010). Importantly, rhesus monkeys possess a parallel polymorphism for *MAOA* (Newman et al., 2005), also X-linked, in which 7 copies of an 18 bp repeat confers low transcriptional activity, and 5 or 6 copies confer high activity. This ortholog makes rhesus monkeys an excellent animal model for studying effects of *MAOA* genotype on phenotypic outcomes (e.g., Karere et al., 2009).

The goal of the present study was to examine the effects of ketamine exposure throughout the prenatal period on measures of activity, emotionality, recognition memory, and response to novelty in rhesus monkey infants, and to determine whether effects were moderated by *MAOA* genotype. We used a sample of infant monkeys that had been exposed to ketamine for colony management reasons, and hypothesized that individuals possessing the low-activity genotype might be particularly susceptible to ketamine's effects.

Methods & Materials

Subjects & Living Arrangements

Subjects were 82 (31 male) infant rhesus monkeys (*Macaca mulatta*), that were 90–126 (mean=105.1) days of age at time of behavioral testing. Animals were drawn from a larger sample if they: 1) were born into the California National Primate Research Center's (CNPRC) indoor time-mate breeding colony, in which adult animals are housed individually until they are paired briefly for mating, which results in precise knowledge of conception date; 2) had exposures to ketamine (either prenatal or postnatal) that were only for routine health checks, minor medical procedures (e.g., dental cleaning for mothers), or ultrasound examination of fetal development; and 3) had participated in CNPRC's BioBehavioral Assessment (BBA) program at 3–4 months of age (see below). Once born, infants and their mothers lived in standard-sized (.58 × .66 × .81 m, Lab Products, Maywood, NJ) indoor housing cages. Mean pairwise relatedness was 1.4% based on subjects' pedigree data that were 1–7 generations deep.

Ketamine Exposures

Ketamine was administered at a dose of 10 mg/kg, intramuscularly; for one animal, a second, smaller dose was given to sustain sedation. Injection was accomplished by briefly restraining the animal using the intra-cage squeeze mechanism, and injecting into a thigh muscle. During the four-year period in which we accrued cases for this study, ketamine was purchased from several vendors, and under different brand names (keta-ject, vetaket, ketaset, ketaved). All products, however, consisted of racemic mixtures of ketamine HCl. The typical 165 day gestation was divided into 55-day trimesters, and the number of ketamine exposures for each trimester was determined. Altogether, animals experienced a mean of 4.54 prenatal exposures to ketamine (range: 1–9). Mean number of exposures in the three trimesters was 2.95 (range: 1–7, SD: 1.08), 0.94 (range: 0–2, SD: 0.64), and 0.65 (range: 0–3, SD: 0.76) exposures, respectively. Postnatally, 23 of the 82 subjects had a single exposure.

Behavioral Assessments

Infants were assessed in CNPRC's BBA program (Capitanio, Mason, Mendoza, Del Rosso & Roberts, 2006; Golub, Hogrefe, Widaman & Capitanio, 2009). Briefly, mothers were lightly anesthetized and the infants were removed and relocated to a part of the facility that was out of auditory or olfactory range of the mothers. Infants, which were always tested in cohorts of 5–8 animals, arrived at 0900 hrs and were individually housed in holding cages (dimensions as above) until they were returned to their mothers at 1000 hr the next day. Each holding cage contained a cloth diaper, a stuffed terry cloth duck and a novel manipulable object (see below). Infants were provided with water, a fruit-flavored drink, commercial monkey chow, and fresh fruit. During the 25-hr BBA period, each infant experienced a variety of assessments, some of which were conducted in the holding cage, and others involved transferring the animal to a test cage in an adjacent room. Behavior was coded using The Observer software (Noldus, 1991); intra- and inter-observer reliabilities for behavioral data collection were checked regularly and exceeded 85% agreement. Data from the following assessments were used in this report:

Behavioral Responsiveness—Each animal was observed for two 5-min periods, once approximately 15 min after placement in the holding cage (0915 hr) on Day 1, and again at 0700 hr on Day 2. The observer sat in front of the cage at a distance of approximately 2.4m avoiding eye contact with the animal. Behaviors were scored according to the ethogram in Golub et al. (2009, Table 2). Behavior frequencies were converted to a rate per 60 sec, and durations were converted to proportion of total time observed. Data from the larger program (more than 1400 animals) were treated to exploratory and confirmatory factor analyses (reported in Golub et al., 2009), and two factors were identified: “Activity” comprised the proportion of time locomoting; proportion of time animals were not in a hang position from the side or top of the cage; rate of environmental exploration; and whether animals ate food, drank water, and were in a crouched posture; and “Emotionality” comprised rate of vocalizing [coos and barks]; and whether the animal displayed threats, lipsmacks, and self-scratch. Scales for both the Day 1 data and the Day 2 data were constructed by summing z-scores for the relevant items, and then re-z-scoring the scales. Day 1 values principally reflect initial responsiveness to the separation/relocation, and Day 2 values reflect adaptation to the test situation.

Novel Object Interaction—Each holding cage contained a novel object, a hollow black PVC cylinder (3.8 cm diameter × 8.9 cm long weighing 0.09 kg) that contained a device (Actiwatch, Philips Respironics, Andover, MA, USA) that recorded whenever a force was exerted on the object. The object was removed one hour before lights went out, at approximately 1615 hr. Data from the recorders were parsed into 5-min blocks of time, and

within each block, the number of 15-sec intervals during which any force was exerted on the objects was counted. (Values resulting from inadvertent contacts owing to removal or return of the animal for other behavioral testing during the day were removed.) Data were summarized as the mean number of 15-sec intervals of contact per 5-min block (maximum score of 20) for two time periods: beginning at initial entry to the cage at 0900 hr until approximately 1230 hr (period 1); and from 1230 hr until removal at 1615 hr (period 2).

Preferential Look—This test of visual recognition memory requires no pre-training and has been found to differentiate normal monkeys from those with damage to limbic structures, prenatal exposure to methyl mercury, and animals from high-risk pregnancies and births (Bachevalier, Brickson & Hagger, 1993; Gunderson, Grant-Webster, Burbacher & Mottet, 1988; Gunderson, Grant-Webster & Fagan, 1987). At 1130 hr, each animal was relocated to a test cage (.39 × .41 × .46 m) that was positioned .69 m from a video monitor (Panasonic KV32540, Secaucus, NJ, 78.7 cm diagonal screen) that was connected to a DVD playback deck (Panasonic DVDS27S), a digital mixer (Panasonic WJMIX30), and a DVD recorder (Panasonic DMRT3040). A low light camera (KTL KPC-S20P, Northern Video, Rocklin, CA) was centered on the viewing monitor to record the animals' responses. The stimuli, which had been pre-recorded onto DVD, comprised seven problems, each of which included the following components: 5-sec of blank screen, 20-sec familiarization trial (a single picture was presented as two identical images side-by-side separated by 25.4 cm of white space), 5-sec blank-screen, 8-sec test trial (in which one of the images was replaced with a novel picture on either the left or right, determined randomly), 5-sec blank-screen, a second 8-sec test trial (same two images in reversed positions). A 100-Hz tone was presented 250 milliseconds prior to trials in order to orient the animal. All stimuli were photographs of rhesus monkeys that were unfamiliar to the subjects (available from the first author). The principal measure was the proportion of time that the animals looked at the novel stimuli divided by the proportion of time animals looked at either the novel or familiar stimuli. Because animals were unrestrained for this test, however, they could choose to look at the stimuli or not. Consequently, we scored a "balk" for a problem if an animal did not look at the stimulus during a familiarization trial, or if the animal did not look at either the novel or familiar stimulus during both test trials of a problem. The number of balks was a second measure of interest.

Genotyping

Genomic DNA was extracted from 100 μ l of whole blood by an alkaline lysis procedure (Sancristobal-Gaudy, Renand, Amigues, Boscher, Levéziel & Bibé B, 2000). Genotyping of the repeat polymorphisms in the promoter region of *MAOA* (Newman et al., 2005) was by PCR with primers MAOA-Forward: CAGAAACATGAGCACAAACG (FAM labeled), MAOA-Reverse: TACGAGGTGTCGTCCAAGTT. The 15 μ l PCR reaction contained 2 μ l of DNA extract as template, 1X PCR Buffer IV without $MgCl_2$ (ABgene-0289, Thermo Fisher Scientific, Rockford, IL, USA), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1.5 M Betaine (Sigma-Aldrich, St. Louis, MO, USA), 0.67 μ M each MAOA primer and 0.5U Taq polymerase (Denville Scientific Inc., Metuchen, NJ, USA). Cycling conditions were: one cycle of 95°C for 5 min, 40 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were diluted 1/10 with water prior to separation by capillary electrophoresis on ABI 3730 DNA Analyzers (Applied Biosystems, Foster City, CA, USA). Fragment size analysis and genotyping were done with STRand software (<http://www.vgl.ucdavis.edu/informatics/strand.php>). *MAOA* alleles were classified according to repeat number of 5 (240 base pairs), 6 (258 bp), and 7 (276 bp). Animals were classified as "low-activity" (n=18) for *MAOA* genotype if they were 7/7 homozygous (female) or 7/- hemizygous (male), and were classified as "high-activity" (n=64) for all other genotypes (Newman et al., 2005). Analysis of *MAOA* genotype

frequency revealed that this sample is in Hardy Weinberg Equilibrium for this gene ($p>0.9$). Average allele frequency was 0.293 (5-repeat), 0.293 (6-repeat), and 0.414 (7-repeat).

Statistical Analysis

Multiple regression was used to determine the separate and joint effects of prenatal ketamine exposure and *MAOA* genotype on outcome measures. The first step included genotype (0=high-activity, 1=low-activity genotypes, see previous paragraph), and the number of ketamine exposures in each of the three trimesters of fetal life. Additional covariates on this step included sex (0=female, 1=male) and number of postnatal ketamine exposures. Step 2 comprised the interaction terms of genotype by number of ketamine exposures for each trimester (three terms total). This was the step of interest. If the change in R^2 from the previous step to this was significant, we examined the significance of the regression coefficients for the interaction terms. (We also describe significant coefficients on Step 2 when the step as a whole was nonsignificant, but consider these only as trends.) Finally, we included in our analyses two additional steps, which we interpret cautiously owing to the size of the model compared to our sample size: Step 3 included the terms reflecting the interaction of ketamine exposures at each trimester (three two-way terms and one three-way term), and Step 4 included interaction terms of genotype by each term included in Step 3. All main effect (Step 1) variables were mean-centered to improve interpretation of lower-order effects when testing interaction effects. A significance level of .05 using two-tailed tests was used to judge significance. Figures depicting significant Step 2 interactions were created as described in Aiken & West (1991).

Results

Few effects were found for genotype alone, and none was found for number of ketamine exposures alone. Several significant interaction effects were found, however, all of which suggested that the impact of prenatal ketamine exposure was greatest for animals with the low-activity *MAOA* genotype. Effects were found primarily for the first two trimesters; regression equations (with standardized coefficients) for significant effects are presented in Table 1; unstandardized coefficients are presented online as Supplementary Information (Table S1).

Behavioral Responsiveness

For animals with the low-activity *MAOA* genotype, prenatal ketamine exposure resulted in altered behavioral responsiveness, with effects most evident for the initial (Day 1) responses to the separation/relocation. For Day 1 Activity, addition of the interaction terms on Step 2 resulted in a significant increment in R^2 ($F(3, 72)=4.763$, $p=.004$); adjusted R^2 for the model was .143. Monkeys with the low-activity *MAOA* genotype showed lower Activity overall in response to the separation/relocation ($\beta=-.235$, $p=.040$), but this response was also dependent on the number of ketamine exposures in the 2nd trimester: a significant regression coefficient for the interaction term ($\beta=0.371$, $p=.001$) indicated that, for animals with the low-activity *MAOA* genotype, increasing exposure to ketamine resulted in increased Activity levels that rose to those seen for animals with the high-activity genotype, who were unaffected by ketamine exposure (Figure 1). For Day 1 Emotionality, addition of the Step 2 variables resulted in a significant change in R^2 ($F(3,72)=3.908$, $p=.012$); adjusted R^2 for the model was .067. A significant regression coefficient for the interaction term of genotype by 1st trimester exposure ($\beta=-.236$, $p=.036$) showed that ketamine exposure again had little effect for animals with the high-activity genotypes, but for those with the low-activity genotype, increasing ketamine exposure led to decreasing expressions of Emotionality (Figure 2).

For the Day 2 measures of responsiveness, effects were seen only for Activity, and they were evident from a significant Step 3 ($F(4, 68)=2.530$, $p=.048$; adjusted R^2 was .057), which comprised the interaction terms of the three trimester variables. One regression coefficient involving genotype was significant on this step: animals with the low-activity genotype that received more ketamine in the 3rd trimester showed greater Activity on Day 2 ($\beta=.291$, $p=.036$). This effect was nearly significant on Step 2 ($p=.056$) as well.

Novel Object Interaction

Animals that possessed the low-activity *MAOA* genotype and had greater ketamine exposure in the 1st trimester showed less contact with the novel object, while more ketamine exposure for animals with the high-activity genotypes resulted in some increase in contact. This was especially evident for Period 2 (Figure 3), in which addition of Step 2 resulted in a significant increment in R^2 ($F(3,72)=3.872$, $p=.013$); adjusted R^2 for this model was .136; for Period 1, Step 2 was not significant ($p=.130$). As indicated, for both Periods, the regression coefficients for the genotype by 1st trimester exposure variable were significant (Period 1 $\beta=-.257$, $p=.024$; Period 2 $\beta=-.317$, $p=.004$). In addition, more object contact in Period 1 was significantly associated with receiving no ketamine in the postnatal period ($\beta=-.250$, $p=.027$), and for Period 2, with being male ($\beta=.296$, $p=.009$) and with having the high-activity *MAOA* genotype ($\beta=-.300$, $p=.010$).

Preferential Look

Across all seven problems, animals showed the expected preference for the novel stimulus (mean proportion of time spent looking at the novel stimulus was 0.576) and number of barks ranged from 0 to 5 (mean=0.61). Step 2 of the regression analysis was significant for number of barks ($F(3,72)=2.826$, $p=.045$; adjusted $R^2=.049$), but not for looking time ($p=.447$). For animals with the high-activity *MAOA* genotype, ketamine had no effect on bark frequency, but for animals with the low-activity genotype, increasing exposure to ketamine during the second trimester was associated with greater balking (Figure 4), as indicated by a significant regression coefficient ($\beta=.247$, $p=.031$).

Discussion

The present study found that a variety of measures examined in infant rhesus monkeys were influenced by prenatal exposure to ketamine, but the effects of ketamine exposure were moderated by *MAOA* genotype: animals that possessed the low-activity genotypes (7/7 for females and 7/- for hemizygous males) were impacted by prenatal ketamine exposure, while animals with the high-activity genotypes were generally unaffected. Moreover, the effects were evident early in prenatal development, with most effects found in the first and second trimesters. We recognize that these results should be confirmed in a replication sample, and consequently consider these results as provisional. Below, we discuss the nature of the behavioral effects found in our study, the prenatal timing of the effects that we found and the role of monoamines, and conclude by speculating that the crossover interactions that we demonstrate have implications for ketamine's mitigating the possibly deleterious effects of possessing a low-activity *MAOA* genotype.

Behavioral consequences of prenatal exposure include affective responses

Administration of NMDA receptor antagonists, such as ketamine, in the late prenatal or early postnatal period has been found to result in deficits in learning and memory (Mickley et al., 1998; Mickley, Remmers-Roeber, Crouse & Peluso, 2000; Fredriksson, Archer, Alm, Gordh & Eriksson, 2004), which can last, in some cases, into adulthood (Mickley et al., 2004). While most of this work has been done in rodents, a recent study with rhesus monkeys, exposed to 24-h of ketamine anesthesia on postnatal days 5 or 6 and tested

through late adolescence, showed similar results (Paule et al., 2011). Our results, in contrast, primarily reflect affective responses: increasing exposure to ketamine (at least for the animals with the low-activity genotype) in the first trimester led to a pattern of results suggesting behavioral inhibition (reduced Emotionality, and reduced contact with novel objects), and increasing exposure in the second trimester was associated with elevated Activity. Importantly, we found no differences in performance in the preferential look task based on ketamine exposure; rather, the difference was in the number of problems contacted (balks), with greater prenatal ketamine exposure in the second trimester associated with more balking. While there were significant methodological differences between our study and those of others (e.g., amount and duration of ketamine exposure), our results are not entirely unexpected. Others have shown effects of ketamine on anxiety-related and activity behaviors (Medeiros et al., 2011; Mickley et al., 2004), and the monkey study described earlier also reported deficits in motivation (Paule et al., 2011). We reiterate, however, that unlike other studies, ketamine exposure per se had no effect on any of our measures; rather, the consequences of ketamine exposure were dependent on MAOA genotype. This may be a significant variable to consider in disentangling the effects of ketamine on affective versus cognitive outcomes.

Ketamine exerts effects at times when monoaminergic systems are developing

Previous research on the effects of ketamine on brain and behavioral development focused on the late prenatal and early postnatal time points for ketamine exposure. The rationale for selecting these times relates to the well-known role of the NMDA receptor in synaptogenesis during the brain growth spurt (Scheetz & Constantine-Paton, 1994). While this growth spurt occurs postnatally in rodents, in monkeys more of it occurs prenatally (Dobbing & Sands, 1979; Gaspar, Cases & Maroteaux, 2003). We found only one marginal effect (on Day 2 Activity) in the third trimester; rather, our effects were evident for exposures in the first and second trimesters.

As described earlier, brain monoamine systems develop early in primates, with these neurotransmitters primarily playing a trophic role in brain development (Nguyen et al., 2001). In addition to neurotransmitters, monoamine oxidases are involved in brain development as well, although their role is not well understood; evidence suggests, for example, that mice lacking the genes for *MAOA* and *MAOB* show reduced proliferation of neural stem cells in the telencephalon during embryonic development as well as significantly elevated serotonin levels (Cheng et al., 2010) and increased anxiety-like and aggressive behaviors as adults (Chen, Holschneider, Wu, Rebrin & Shih, 2004; Cases et al., 1995). To the extent that parallel neural processes are operating in monkeys with the low-activity *MAOA* genotype, one would expect higher levels of monoamine neurotransmitters in these animals; importantly, ketamine exposure has been shown to increase serotonin levels in rat brains (Kari et al., 1978). It's possible that there are additive effects of MAOA genotype and ketamine exposure that could lead to elevated monoamine levels which, at least for serotonin, can have negative consequences for neural and behavioral development (Gaspar et al., 2003; Ansorge, Zhou, Lira, Hen & Gingrich, 2004). The precise role that prenatal ketamine exposure can play in these processes remains to be identified, however.

Can ketamine mitigate the potential negative consequences of a low-activity genotype?

Examination of our figures reveals that all of the genotype by exposure interactions that we report are crossover interactions; that is, at a certain number of exposures, the responses of the animals with the low-activity genotype are indistinguishable from those with the high-activity genotype. This raises the intriguing possibility that ketamine might be able to mitigate the psychological "risk" that has been found for individuals who possess the low-activity genotype and who experience adverse early experiences. A critical aspect of this use

would be dosage, however; as described earlier, microdialysis studies show that ketamine's effects are dose-dependent (Moghaddam et al., 1997). In fact, anti-depressant efficacy has been seen with a single intravenous dose of 0.5 mg/kg, a dose that is subanesthetic and lower than the 10 mg/kg im used in our animals (and substantially less than the 24-hr exposures used in the studies modeling pediatric anesthesia). Further study in this animal model might reveal whether comparable behavioral effects could be seen with a lower dose in monkeys with the low-activity genotype.

Limitations and Conclusion

We recognize several limitations of this study. Most importantly, ours was a retrospective study that utilized healthy pregnant females that had received ketamine for anesthetic purposes at no pre-specified points in gestation. Tighter control of the timing of exposures, done in a prospective fashion, would be preferred. In addition, we note that every animal in our study had at least one exposure. Given the common usage of ketamine as a veterinary anesthetic, we suspect there are few, if any, animals at our (or others') primate facilities that have not experienced a prenatal ketamine exposure. Prospective follow-up work would benefit from having animals in a no-exposure, control, condition. Similarly, most animals in the present sample were exposed to ketamine in more than one trimester; while multiple regression can produce statistical control of exposure number, subsequent work should employ experimental control. Finally, the present study does not provide information on the mechanisms by which ketamine exposure results in affective disturbance among animals with a low-activity *MAOA* genotype. For example, we do not know whether these effects are mediated by direct effects of ketamine on specific receptors in the fetal brain or whether the effects are more indirect, mediated, possibly, by ketamine's impact on other processes, such as maternal/placental physiology. Despite these limitations, however, the relatively large sample size and the precise knowledge of conception dates and gestational ages of exposure in our study increase confidence that there are indeed effects of prenatal ketamine exposure, which vary with *MAOA* genotype, on measures relating to affect. This unique result contributes to a growing literature on personalized medicine, and suggests that monoamine genotype should be considered an important variable when considering the prenatal effects of psychotropic substances on later behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
MAOA genotype interacts with number of ketamine exposures in the second trimester for Day 1 Activity.

Figure 2.
MAOA genotype interacts with number of ketamine exposures in the first trimester for Day 1 Emotionality.

Figure 3. *MAOA* genotype interacts with number of ketamine exposures in the first trimester for mean number of 15-second intervals of contact with a novel object per 5-minute block. Data for both sexes are combined for this figure.

Figure 4. *MAOA* genotype interacts with number of ketamine exposures in the second trimester for the number of balks in the preferential look test.

Table 1

Standardized regression coefficients for outcome measures. Change in R² upon addition of interaction terms is indicated in last line.

Assessment: Measure:	Behavioral Responsiveness		Novel Object Contact		Preferential Look
	Activity	Emotionality	Period 1	Period 2	Balks
Independent Variables:					
MAOA - low activity=1	-.235 *	.032	-.106	-.300 **	.036
1 st trim ket exposures	.185	-.148	.145	.140	.022
2 nd trim ket exposures	.062	.024	-.114	.073	.077
3 rd trim ket exposures	.131	.128	.097	-.031	.107
sex (male=1)	.200	-.074	.120	.296 **	-.002
postnatal ket exposures	-.080	.084	-.250 *	.052	-.030
MAOA × 1 st trim	-.110	-.236 *	-.257 *	-.317 **	-.130
MAOA × 2 nd trim	.371 ***	-.173	.053	.192	.247 *
MAOA × 3 rd trim	-.153	-.223(.053)	-.070	-.009	.163
R ² change for Step 2:	.151 **	.135 *	.068	.124 *	.100 *

* p<.05;

** p<.01;

*** p<.001