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Activation of the Maternal Immune System During Pregnancy Alters Behavioral Development of Rhesus Monkey Offspring

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Abstract

Background: Maternal infection during pregnancy is associated with an increased risk of schizophrenia and autism in the offspring. Supporting this correlation, experimentally activating the maternal immune system during pregnancy in rodents produces offspring with abnormal brain and behavioral development. We have developed a nonhuman primate model to bridge the gap between clinical populations and rodent models of maternal immune activation (MIA).

Methods: A modified form of the viral mimic, synthetic double-stranded RNA (polyinosinic:polycytidylic acid stabilized with poly-L-lysine) was delivered to two separate groups of pregnant rhesus monkeys to induce MIA: 1) late first trimester MIA ($n = 6$), and 2) late second trimester MIA ($n = 7$). Control animals ($n = 11$) received saline injections at the same first

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or second trimester time points or were untreated. Sickness behavior, temperature, and cytokine profiles of the pregnant monkeys confirmed a strong inflammatory response to MIA.

Results: Behavioral development of the offspring was studied for 24 months. Following weaning at 6 months of age, MIA offspring exhibited abnormal responses to separation from their mothers. As the animals matured, MIA offspring displayed increased repetitive behaviors and decreased affiliative vocalizations. When evaluated with unfamiliar conspecifics, first trimester MIA offspring deviated from species-typical macaque social behavior by inappropriately approaching and remaining in immediate proximity of an unfamiliar animal.

Conclusions: In this rhesus monkey model, MIA yields offspring with abnormal repetitive behaviors, communication, and social interactions. These results extended the findings in rodent MIA models to more human-like behaviors resembling those in both autism and schizophrenia.

Keywords

Animal model; autism spectrum disorder; immune activation; macaque; nonhuman primate; poly IC; schizophrenia

Autism spectrum disorder (ASD) and schizophrenia (SZ) are chronic and disabling brain disorders that each affect approximately 1% of the population (1,2) and are thought to be caused by complex interactions between genetic and environmental factors (3-5). Recent evidence suggests that the prenatal environment, and particularly the maternal immune environment, plays a critical role in some cases of ASD and SZ (6-8). Epidemiologic studies reveal that women exposed to viral, bacterial, or parasitic infections during pregnancy have an increased risk of having a child that later develops SZ (9-14). Likewise, maternal viral and bacterial infections are associated with an increased risk of ASD in the offspring (15-19). The diversity of maternal infections associated with ASD and SZ outcomes suggests that the maternal immune response is the critical link between sickness in the mother and altered neurodevelopment in her child.

Understanding the mechanism by which maternal immune activation (MIA) during pregnancy increases the risk for SZ and ASD is essential to developing novel preventative or therapeutic strategies. Rodent models have identified molecular, cellular, and behavioral abnormalities associated with prenatal immune challenge (20). Maternal influenza infection (21-24) or injection of the bacterial endotoxin lipopolysaccaride (25-27) yields offspring with behavioral abnormalities, neuropathology, and altered gene expression that are relevant to both SZ and ASD. Similar outcomes are obtained by treating pregnant rodents with the viral mimic, synthetic double stranded RNA (polyinosinic:polycytidylic acid [poly IC]), which stimulates an inflammatory response in the absence of a specific pathogen (28). Offspring born to pregnant dams treated with poly IC at mid-gestation demonstrate repetitive behaviors and deficits in social and communication behaviors that resemble features of ASD, as well as elevated anxiety, deficits in prepulse inhibition, latent inhibition, and working memory that resemble clinical features of both ASD and SZ (21,29-32). Neuropathology observed with ASD (localized loss of Purkinje cells) and SZ (enlarged ventricles) have been reported in poly IC rodent models (33-35), and there are numerous other alterations in brain structure, neurochemistry, gene expression, and immune function

(36-39). The deleterious effects on brain and behavior in the mouse MIA model appear to be mediated by the maternal cytokine response, in particular interleukin-6 (40).

While rodent models have laid the foundation for understanding the effects of MIA on fetal brain development, these models have limitations. Extrapolating the timing of fetal brain development between rodents and humans is complicated by the fact that the neural events of the human third trimester occur during the early postnatal period in rodents (41). Moreover, there are challenges in relating the rodent brain to the human brain and rodent behavior to human behavior. This is particularly problematic for disorders such as ASD and SZ that are characterized by deficits in a range of complex cognitive, social, and affective functions. Indeed, portions of the human brain, such as prefrontal cortex, which mediate these functions and are heavily impacted in ASD and SZ, are poorly developed in the rodent brain (42). Understanding human disorders involving higher cognitive functions will benefit from studies in animal species more closely related to humans. Nonhuman primates, such as rhesus macaques (*Macaca mulatta*), demonstrate many features of human physiology, anatomy, and behavior, making them an appropriate species to study a variety of human brain disorders (43). The rhesus monkey lives in a complex, hierarchical social system and uses many forms of human-like communication such as facial expressions and social gestures (44). The rich social and cognitive repertoire of rhesus monkeys provides a framework to relate behavioral changes observed in the animal model more directly to human mental illness.

We have developed a novel, nonhuman primate model using a modified form of the viral mimic poly IC, which is adapted for use in primates (polyinosinic:polycytidylic acid stabilized with poly-L-lysine [poly ICLC]). This synthetic RNA is recognized as foreign by the primate immune system and induces a transient innate inflammatory response (45,46). Pregnant rhesus monkeys were injected with poly ICLC over a 72-hour period at the end of the first or second trimester. These gestational ages were selected based on human epidemiologic data identifying the first and second trimesters as vulnerable time points where exposure to MIA increases the risk of autism and schizophrenia (14,17). We evaluated sickness behavior, body temperature, and cytokine responses in the dams to confirm a strong immune activation and then analyzed the behavioral development of the offspring for 4 years. Here, we present our initial behavioral findings through 24 months of age, documenting the emergence of abnormal behavior in rhesus offspring exposed to MIA.

Methods and Materials

All experimental procedures were developed in consultation with the veterinary staff at the California National Primate Research Center. Protocols were approved by the University of California, Davis Institutional Animal Care and Use Committee. Detailed methods are provided in Supplement 1.

Maternal Administration of Poly ICLC

Twenty-four multiparous rhesus monkeys were assigned to one of three experimental groups: 1) first trimester MIA (MIA^1), 2) second trimester MIA (MIA^2), or 3) saline control animals (CON^{Saline}) (Table S1 in Supplement 1). Pregnant animals in the MIA groups were

injected with .25 mg/kg synthetic double-stranded RNA (poly ICLC) (Oncovir, Inc., Washington, DC) via intravenous injection while restrained by trained technicians on gestational days 43, 44, and 46 (MIA^1) or 100, 101, and 103 (MIA^2).

Rearing Conditions

Infants were raised in individual cages with their mothers, where they had visual access to other animals at all times. For 3 hours each day, one adult male and four familiar mother-infant pairs were allowed to freely interact in a large cage to provide enrichment and facilitate species-typical social development. Each group consisted of a mixture of male and female offspring of both MIA and control experimental groups. The infants were weaned from their mothers at 6 months of age but continued the same socialization routine.

Behavioral Observations

Behavioral data were collected throughout the first 2 years of life using our standardized rhesus developmental battery (Table S2 in Supplement 1) (47-50). For the sake of brevity, only behavioral assays associated with significant results are presented (Table 1). Unless noted in the material description in Supplement 1, behavioral data were collected using focal animal samples (51) in a predetermined, pseudo-random order, employing a catalog of behaviors commonly used for this species (Table S3 in Supplement 1). Behaviors initiated or received by the focal animal, as well as the behavior of other animals (i.e., mother, other adults, peers) toward the focal animal were recorded, resulting in the quantification of mother-infant and peer social interactions throughout development.

Statistical Analysis

Preliminary analyses revealed that the behavioral profiles of the saline-treated monkeys and the untreated control monkeys were very similar. They were therefore pooled to form a single control group. Mixed-effects linear models (52) were used to analyze the frequency and duration of the behaviors, since all the experiments involved repeated observations. Suitable transformations were performed for the variables that violated the assumption of normality. All core models included fixed effects for group (MIA^1, MIA^2, and control) and gender (to adjust for gender imbalance across groups and account for its potential effect on frequency and duration of the behaviors) and a random effect for animal (to account for the correlated nature of the data). For experiments involving stimulus monkeys or where time effects were detected, additional fixed terms (for stimulus monkey gender, time, interaction of time with group, etc.) were also added to the core model and tested. These terms were retained in the models only if they were significant. All tests were two-sided, with $\alpha = .05$.

Results

Sickness behavior, temperature and cytokine profiles of the pregnant monkeys confirmed a strong inflammatory response to poly ICLC (Figures S1 and S2 and Tables S4-S7 in Supplement 1). For the sake of brevity, only significant behavioral results from the offspring are presented in detail. There were no consistent differences across offspring in physical growth, motor or reflex development, adrenal activity, interactions with mothers, or

development of threat detection in the first 6 months of postnatal life (Table S8 in Supplement 1).

Mother Preference

Following weaning at 6 months of age, MIA offspring differed from control animals during a test designed to evaluate infant attachment to the mother. While all animals, irrespective of treatment condition, demonstrated a species-typical attachment to their own mother, we detected differences in the patterns of the animals' responses to the test. Offspring in the MIA^2 treatment group produced significantly more distress/self-soothing behaviors that are commonly observed during the attachment assay (i.e., tantrums, convulsive jerk, self-clasp, infant crook tail) than MIA^1 or control offspring. Group differences were not apparent on the first day but emerged over the 4 consecutive days of testing (Figure 1; Table 2; Figure S9 in Supplement 1). On the final day, MIA^2 treatment offspring were highly reactive and control offspring were moderately reactive, while MIA^1 treatment offspring displayed almost no evidence of reactivity.

Solo Observations

At 10 months of age, we conducted postweaning solo observations of the animals alone in a large cage to screen for abnormal motor stereotypic and/or self-directed behaviors that are common to captive rhesus monkeys (see Table S1 in Supplement 1 for definitions) (53). Compared with control animals, the MIA^2 animals produced motor stereotypic and/or self-directed behaviors more frequently than control animals ($p = .002$) (Figure 2A; Table 3). First trimester MIA animals displayed a trend level increase in these behaviors compared with control animals ($p = .06$). We also detected trend level differences in the frequency of affiliative contact "coo" calls produced by the MIA^1 offspring when observed alone in the large cage ($p = .08$). Juvenile solo observations were repeated at 22 months of age. Both MIA groups produced significantly more motor stereotypic and/or self-directed behaviors than control animals ($p = .03, .01$, respectively) (Figure 2A; Table 3). As observed in the postweaning period, MIA^1 offspring produced fewer affiliative contact coo calls than control animals, although the difference remained at trend level. At this later time point, however, MIA^2 offspring produced significantly fewer coo calls than control animals (Figure 2B, Table 3).

Interaction with Novel Conspecifics (Y-Maze)

At 17 months of age, we conducted an exploratory assay designed to evaluate social interactions with an unfamiliar conspecific, using a Y-shaped testing chamber in which the experimental animal had access to two chutes. A novel stimulus animal was housed in a holding cage at the end of one chute; the other arm led to an empty cage. While there were no differences in the amount of time spent in the social versus nonsocial arms of the cage (Table 4) and there were few interactions with the novel animal, we did detect differences in coo vocalization and repetitive behaviors (Table 4). While there were no differences in the total number of coo vocalizations, the MIA^1 offspring exhibited a trend level difference in the frequency of affiliative contact coo calls produced when alone in the nonsocial arm of the Y-maze ($p = .06$). Paralleling the results from postweaning and juvenile experiments, the

MIA^2 offspring produced significantly more motor stereotypic and/or self-directed behaviors than control animals (p = .002; Figure 2B).

Interaction with Novel Conspecifics (Two-Chamber Social Approach)

This test was modeled after the sensitive assay of sociability used for mouse models of ASD (54-57). All subjects, irrespective of experimental condition, spent significantly more time in the social chamber than in the nonsocial chamber (Table 5). The MIA^1 offspring, however, differed from control animals in several behavioral measures. They produced fewer total affiliative contact coo calls (Figure 2B, Table 5), and they approached the stimulus cage more frequently than control animals and initiated proximity with the unfamiliar animal more than twice as frequently as the control animals (Figure 2C, Table 5). Differences were also detected in the amount of time spent in contact or proximity (i.e., within arm's reach) of the stimulus cages within the chambers. Compared with control subjects, both MIA groups spent more time near the small empty cage in the nonsocial chamber. However, only MIA^1 offspring spent more time near the small cage containing an unfamiliar conspecific in the social chamber (Figure 2C). There were no differences in the frequency of entering or exiting the social and nonsocial chambers or in the frequency of approaching the empty stimulus cage, suggesting that the differences in approach frequency were specific to the social stimulus and not reflective of global changes in activity.

Discussion

Rhesus monkey offspring exposed to MIA in utero differ from control offspring in measures of repetitive behaviors, vocal communication, and social interactions. These alterations in behavior overlap with the core diagnostic domains of ASD, and the latter behaviors may also be relevant for SZ. The development of some abnormal behaviors (increased reactivity in MIA^2 offspring and abnormal social behavior in MIA^1 offspring) depends on the specific period of MIA exposure during pregnancy, while other abnormal behaviors (decreased affiliative vocalizations and increased repetitive behaviors) are present in both MIA groups (Figure S3 in Supplement 1).

While the majority of rodent MIA models have reported behavioral abnormalities in adult offspring, here we describe the emergence of behavior over the first 2 years of life in a nonhuman primate model. This period for rhesus monkeys is roughly equivalent to early childhood in humans. Although group differences were not consistently detected at the early time points, by 2 years of age the MIA monkey offspring began to demonstrate consistent patterns of behavioral changes. The first indication of differences between the experimental groups occurred immediately after weaning, at 6 months of age, during an assessment of emotional attachment to the mother. While all animals, irrespective of treatment condition, demonstrated a species-typical attachment to their own mother, the MIA animals' responses to the test were different from control animals. The MIA^2 offspring displayed a dramatic increase in distress/self-soothing behaviors over the 4-day testing period that was not observed in the control animals. In contrast, the MIA^1 offspring produced almost none of these behaviors. Differences in the animals' responses to the test were most pronounced on the fourth consecutive day of testing, suggesting that this particular repeated assay can reveal

changes in distress/self-soothing behaviors that are not detected in other paradigms. While we do not know why the MIA^2 offspring responded with increased distress/self-soothing behaviors, mouse MIA models also exhibit behaviors indicative of heightened anxiety (i.e., less time in the center of the open field paradigm and reluctance to explore novel objects) that may provide insight into this atypical response in the monkey (21).

Additional behavioral changes in monkey MIA offspring began to emerge during the postweaning (6–12 months) and juvenile (12–24 months) periods. It is important to note that these early changes in behavior were subtle, as there were no group differences detected in daily home cage observations or in weekly observations of the animals interacting with familiar peers. However, when the MIA animals were removed from these familiar environments and observed alone, they consistently produced more motor stereotypic and/or self-directed behaviors than control animals. These behavioral pathologies were most pronounced in the MIA^2 group, as indexed by a high frequency in three different testing paradigms. Animals in the MIA^1 group also appeared to produce more repetitive behaviors than control animals, although these differences did not attain statistical significance until the animals reached 2 years of age. It is well established that restricted rearing environments, small cage size, and stress-inducing events can trigger stereotypies in laboratory animals (53,58,59) and we designed our protocols to minimize these factors. The fact that the control animals exhibited a low frequency of motor stereotypic and/or self-directed behaviors indicates that we can reasonably attribute these behaviors to MIA, rather than general socioenvironmental restrictions. The results from this nonhuman primate model parallel findings of increased repetitive and compulsive behaviors of mouse MIA offspring that exhibit high levels of repetitive behaviors in marble burying and self-grooming tests (29).

When the animals were removed from their home cages where they had constant visual access to familiar animals, we also collected data on any social signals, including vocalizations, that were produced. During these temporary separations, young monkeys often produced affiliative coo calls that are thought to serve the function of reestablishing contact with conspecifics (60-63). Compared with control offspring, both groups of MIA offspring produced fewer coo calls, although only the MIA^2 group differed significantly from control animals under these conditions. Interestingly, the MIA^1 offspring continued to exhibit reduced coo calling when removed from their home cage and introduced to an unfamiliar peer, suggesting that the presence of an unfamiliar animal may differentially impact social buffering for the MIA groups (64,65). The reduced affiliative vocalizations observed in macaque MIA offspring are consistent with data from male MIA mice, which display a reduced number of vocalizations as pups when they are isolated from their littermates and mother and as adults in the presence of a female (29).

Given that impaired social functioning is a hallmark feature of both ASD and SZ, we would expect a valid animal model to also produce impairments in social processing. While MIA offspring did not differ from control animals during daily interactions with familiar peers, group differences were detected during interactions with an unfamiliar social partner, which is considered to be a more challenging social encounter. It is important to point out that the nature of behavioral perturbations in an animal model may be complex and species-specific, especially in challenging social interactions. In mice, for example, the default response to an

unfamiliar conspecific is to approach and investigate. Thus, decreased time spent investigating a novel animal is taken as evidence of diminished sociability (66) and is a common behavioral outcome of MIA mouse models (21,29,40). For rhesus monkeys, the decision to approach and interact with another animal depends on a number of internal (i.e., individual temperament differences) and external (i.e., characteristics of the unfamiliar animal, presence or absence of kin) factors (67-71). For many species of nonhuman primates, immediately approaching an unfamiliar conspecific or behaving impulsively with familiar animals is met with negative outcomes and physical aggression (72-79). The default for rhesus monkeys is to approach an unfamiliar conspecific with caution and after considerable evaluation at a distance. However, when evaluated with an unfamiliar conspecific at 2 years of age, MIA^1 offspring exhibited a clear deviation from the species-typical social protocol for rhesus monkeys by frequently approaching, contacting, and staying within arm's reach of the unfamiliar animal. Thus, both mouse and monkey MIA models result in deviation from species-typical social norms.

Behavioral changes in mouse MIA models have been interpreted as bearing resemblance to features of both ASD and SZ (20,80-82), although the timing of the prenatal challenge likely determines the ultimate consequences of MIA exposure (83-90). The 165-day macaque monkey pregnancy provides an opportunity to further delineate vulnerable periods of gestation during which MIA alters specific neural networks and ultimately leads to distinct behavioral trajectories over a relatively protracted period of postnatal development. Our results indicate that experimentally inducing MIA at either late first trimester or late second trimester produces offspring with overlapping alterations in repetitive behaviors and affiliative vocalizations, as well as distinct changes in reactivity and social interactions. While it is premature to determine if MIA in the primate model is related specifically to ASD or SZ or to more general neurodevelopmental issues (91), we can begin to evaluate the nature and timing of the behavioral outcomes of the monkey MIA model.

Stereotypic behaviors, for example, are one of the diagnostic features of ASD and were consistently observed throughout postnatal development in the MIA^2 offspring and to a lesser extent in the MIA^1 offspring. While these behaviors support the face validity of the model, it is important to recognize that stereotypies are observed in a variety of developmental, psychiatric, and neurological disorders and are not specific to ASD. However, both ASD and SZ are characterized by changes in social cognition and emotion (92), which were also altered in the macaque MIA offspring compared with control animals. While both MIA groups exhibited decreased frequency of the affiliative contact coo calls when observed alone, only the MIA^1 offspring produced fewer coos in a social context. Likewise, only the MIA^1 offspring exhibited inappropriate social interactions with a novel conspecific. We suggest that the inappropriate social approach behaviors observed in the animal model may be reminiscent of the active but odd subtype of social interaction style described in ASD (93) and the complex social functioning impairments in SZ (94). We have initiated an eye-tracking study to evaluate social processing in the monkey model and will utilize these data to further clarify the nature of the social impairments and the relevance to ASD and SZ.

The timing of behavioral alterations is another important consideration. Autism spectrum disorder, for example, is diagnosed in early childhood (95), while the onset of psychotic symptoms of SZ typically occurs during the transition from adolescence to adulthood (96). In the present study, we first detected group differences in response to weaning at 6 months of age, which is roughly equivalent to a 2-year-old child. While this time frame is more consistent with the early symptom onset of ASD, prospective studies of patients who develop SZ also have social and neurocognitive impairments that emerge long before psychiatric SZ symptoms (97-100). Observations of macaque offspring will continue as they mature, which is needed to interpret the emergence of symptoms over time, as well as the long-term effects of MIA in primates and the relevance to human neurodevelopmental disorders.

While the rhesus monkey provides an animal model that closely parallels human brain organization and cognitive and social functioning, there are ethical and pragmatic limitations in the development of a nonhuman primate model. The primary limitation of the current study is the sample size. A second limitation is that we must wait until the conclusion of the behavioral studies (approximately 4 years) before initiating brain pathology studies that are often simultaneously carried out in rodent models. Thus, the data presented here describe behavioral outcomes but do not provide a mechanistic neural basis for the specific abnormalities. Mouse MIA models, however, have identified several plausible mechanisms by which poly IC-induced immune responses can disrupt fetal brain development (101-104). The maternal cytokine response to poly IC, in particular interleukin-6 (40), plays a critical role in triggering immune activation and endocrine changes in the placenta (105) and altered cytokine expression in the fetal brain, as well as long-lasting changes in cytokine expression in the brains of MIA mouse offspring as they mature (36). In the present study, we utilized a modified form of poly IC (poly ICLC), which stimulates comparable inflammatory responses in humans and nonhuman primates (45,46,106). While other nonhuman primate models of MIA have explored maternal immune challenges in the third trimester (107,108), we focused our efforts on the first and second trimesters, as human studies have identified these as the gestational windows of vulnerability for ASD and SZ associated with maternal immune challenge (109). This time frame of early fetal brain development captures the peak period of macaque neurogenesis (110-117). Short *et al.* (107) report that rhesus offspring born to mothers exposed to influenza in the early third trimester demonstrate reduced gray matter volume throughout the cortex and increased white matter in the parietal cortex at 1 year of age. We predict that MIA exposure in the late first and second trimesters also produce changes in brain development of the offspring. We are currently exploring brain pathology in these animals to determine if MIA offspring demonstrate structural or functional brain pathologies characteristic of ASD or SZ and will initiate a comprehensive histological evaluation of the brain at the conclusion of the behavioral studies.

While experimentally inducing MIA in the primate model alters behavioral development, it is important to emphasize that sickness during human pregnancy is not uncommon (118,119), and clearly not all women who experience infection during pregnancy have children later diagnosed with a neurodevelopmental disorder (120). A number of factors, including genetic susceptibility, the intensity of the infection, and the maternal and/or fetal response, as well as the precise timing of the immune challenge, likely influence the degree

to which MIA alters fetal brain development and may ultimately determine which disease phenotype (ASD or SZ) is expressed. With mounting evidence of the increased risk of psychiatric disorders in offspring exposed to MIA, increased efforts to understand MIA-induced alterations in brain development are clearly needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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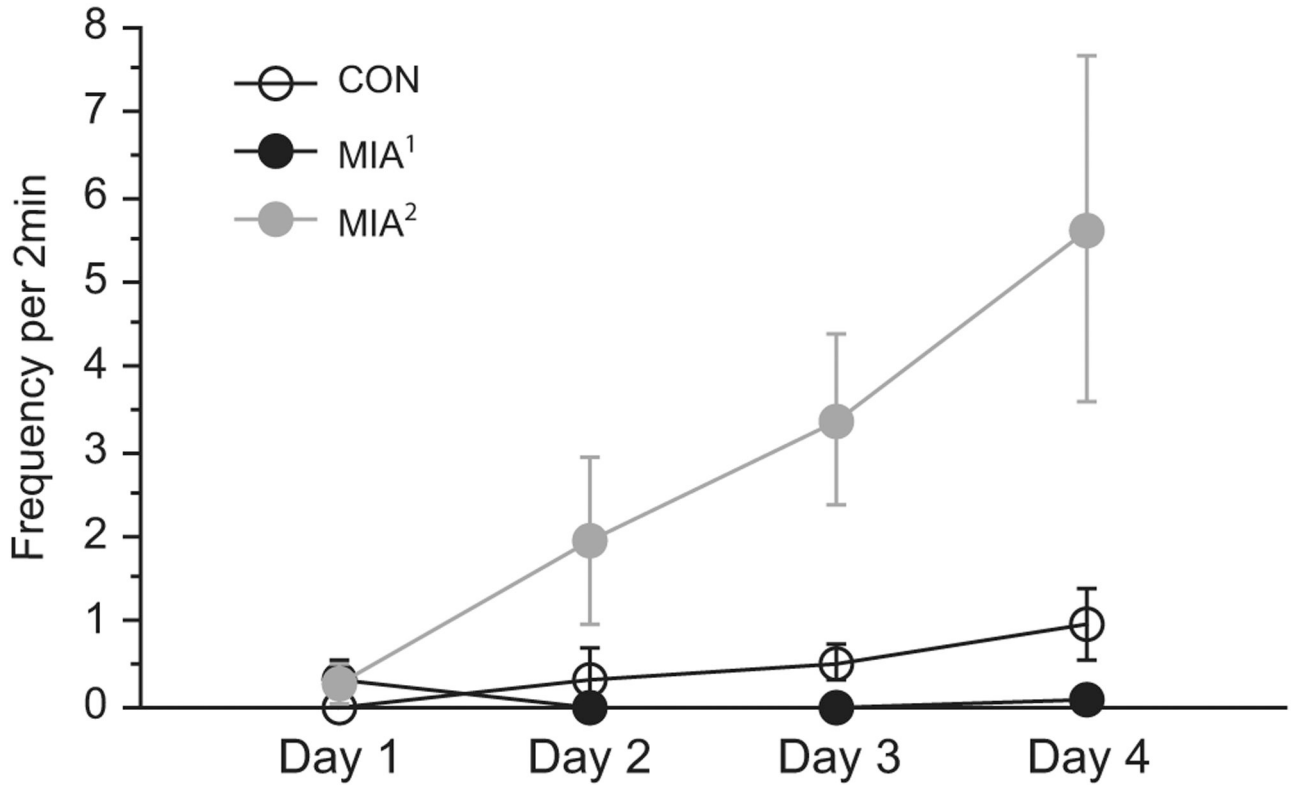

Figure 1.

Maternal immune activation (MIA) offspring exhibit abnormal responses to weaning. Although all animals demonstrate a species-typical attachment to their own mother, MIA offspring exhibit an unusual response in the attachment test. Second trimester MIA (MIA^2) offspring produce significantly more distress or self-soothing behaviors (i.e., tantrums, convulsive jerk, self-clasp, infant crook tail) than control (CON) offspring. This group difference emerges over the 4 days of testing, with both MIA groups showing a different pattern over time than control animals ($p < .001$ and $p < .003$ for the differences in slopes, respectively). Thus, on the final day, MIA^2 offspring are highly reactive, control animals are moderately reactive, and first trimester MIA (MIA^1) offspring display little evidence of reactivity ($p < .01$ for difference from control animals for both MIA^1 and MIA^2 groups on day 4).

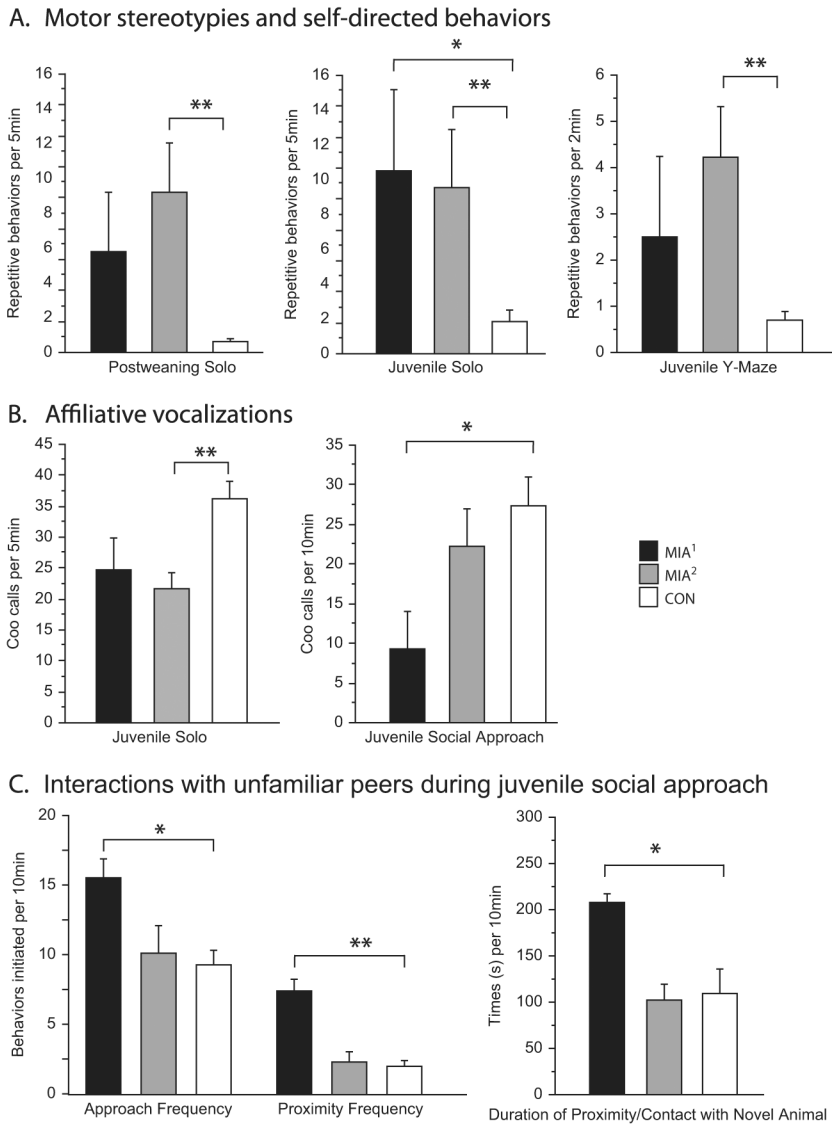

Figure 2.

(A) Maternal immune activation (MIA) offspring exhibit increased frequency of motor stereotypies and self-directed behaviors. Left panel: When observed alone in a large cage at 10 months of age, second trimester MIA (MIA^2) animals produce significantly more repetitive behaviors than control animals (CON) (**$p \leq .01$). The first trimester MIA (MIA^1) offspring also produce more repetitive behaviors than control animals, but this difference does not reach statistical significance at 10 months ($p = .06$). Middle panel: When observed alone at 22 months of age, MIA^1 offspring produce significantly more repetitive behaviors (*$p \leq .05$). Second trimester MIA animals also produce significantly more repetitive behaviors than control animals at 22 months (**$p \leq .01$). Right panel: When tested at 17 months of age in the Y-maze social preference assay, MIA^2 treatment animals produce significantly more repetitive behaviors than control animals (**$p \leq .01$). **(B)** Maternal immune activation offspring display decreased affiliative vocalizations. Left panel: At 22 months, MIA^2 offspring produce significantly fewer coo calls than control animals (**$p <$.

01). Right panel: When observed with a novel conspecific at 24 months of age, MIA[1] offspring produce significantly fewer coo calls than control animals (*$p \leq .05$). **(C)** Maternal immune activation offspring exhibit inappropriate interactions with unfamiliar conspecifics. Left panel: First trimester MIA offspring demonstrate inappropriate social interactions with an unfamiliar animal, as indexed by high frequency of approaching (*$p < 0.05$) and more frequently moving within arm's reach of the unfamiliar animal (**$p < .01$). Right panel: First trimester MIA offspring remained near the unfamiliar animal, as indexed by the duration of time spent in physical contact or within arm's reach of the unfamiliar animal (*$p < .05$)

Table 1.

Behavioral Phenotyping Assays

Behavioral Assay	Brief Description	Relevance to Autism Spectrum Disorders and Schizophrenia
6–12 Months of Age		
Mother preference [a]	Following weaning, each infant was tested for 4 days to evaluate one aspect of mother-infant attachment, the infant's preference for its own mother versus another familiar adult female (12 2-minute trials/subject).	Measures of attachment serve as control parameters for species-typical development and response to separation (48).
Postweaning solo Observations [b]	At approximately 10 months of age, the animals were observed alone in a large, unfamiliar cage for two 5-minute focal samples on 2 separate days to screen for abnormal behaviors such as motor stereotypies or self-directed behaviors.	Solo observations are conducted to screen for a wide array of stereotyped behaviors produced by rhesus monkeys (49,53,58).
12–18 Months of Age		
Juvenile Y-maze	At approximately 18 months of age, animals were given visual access to a novel conspecific in one arm of a Y-maze test apparatus. Each animal was tested for six 2-minute trials on 2 separate days, meeting an opposite-sex conspecific on the first day and a same-sex conspecific on the second day.	Initial social assays with novel conspecifics were carried out using the Y-maze testing apparatus and later followed with the three-chambered social approach assay described below.
Juvenile solo Observations [b]	At approximately 22 months of age, the animals were observed alone in a large, unfamiliar cage for two 5-minute focal samples on 2 separate days to screen for abnormal behaviors such as motor stereotypies or self-directed behaviors.	Solo observations are conducted to screen for a wide array of stereotyped behaviors produced by rhesus monkeys (49,53,58).
Juvenile social approach [c]	At approximately 24 months of age, social interactions with a novel conspecific were evaluated using a modified version of the mouse three-chambered social approach assay (20 minutes/subject).	The high-throughput social approach assay used in mouse models (54) paired with the fine-grained focal observations utilized in our nonhuman primate studies (47,48) provide a screen for sociability as indexed by the amount of time spent in a chamber with a constrained, novel conspecific.

ASD, autism spectrum disorders; SZ, schizophrenia.

[a] Assays used to control for changes in physical development, reflexes, fear response development, maternal attachment, and activity levels that are not directly related to the core features of ASD and SZ.

[b] Behavioral assays targeting repetitive behaviors and restricted interests.

[c] Behavioral assays targeting social and communication domains.

Table 2.

Mother Preference

	Estimate (SE)	*p* Value
Estimated Trajectory for the Control Group		
Baseline (day 1)	−.1 (.1)	.60
Linear change with time (per day)	.2 (.0)	<.001
Estimated Difference between MIA[1] and Control Animals		
Baseline (day 1)	.2 (.2)	.31
Linear change with time (per day)	−.2 (.1)	<.001
Estimated Difference between MIA[2] and Control Animals		
Baseline (day 1)	.3 (.2)	.09
Linear change with time (per day)	.1 (.1)	.003

Summary (parameter estimates and standard errors) of the mixed-effects models assessing the relationship of group and time with frequency of reactive behaviors.[a] Differences from control animals are estimated from mixed-effects regression models fitted to the frequency of behaviors and adjusted for gender, day, and the interaction between group and day.

MIA[1], first trimester maternal immune activation; MIA[2], second trimester maternal immune activation.

[a]The outcome was first transformed using the fourth root to improve its normality.

Table 3.

Behaviors During Postweaning and Juvenile Solo Observations

Behavior	Average Group Frequency			Difference from Control Group			
				MIA[1] vs. Control Group		MIA[2] vs. Control Group	
	MIA[1] Mean (SD)	MIA[2] Mean (SD)	Control Group Mean (SD)	Estimate (SE)	*p* Value	Estimate (SE)	*p* Value
Postweaning							
Coo	27.5 (8.0)	34.6 (9.5)	38.5 (10.9)	−10.2 (5.5)	.08	−3.5 (5.0)	.48
Stereotypy [a]	5.8 (8.3)	9.1 (7.6)	.5 (.7)	1.4 (.7)	.06	2.2 (.6)	.002
Juvenile							
Coo	24.7 (12.1)	21.5 (6.9)	36.1 (9.6)	−8.5 (4.8)	.09	−14.8 (4.4)	.003
Stereotypy [a]	10.5 (11.4)	9.5 (8.9)	1.8 (2.3)	1.7 (.7)	.03	1.8 (.7)	.01

Descriptive statistics and summary (parameter estimates and standard errors) of the mixed-effects models assessing the relationship between group and frequency of behavior variables. Average group behaviors are based on observed frequency of behaviors. Differences from control groups are estimated from mixed-effects regression models fitted to the frequency of behaviors and adjusted for gender.

MIA[1], first trimester maternal immune activation; MIA[2], second trimester maternal immune activation.

[a]Variable square-root transformed to improve its normality.

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Table 4.

Duration and Frequency of Behaviors in Juvenile Y-Maze Paradigm

Behavior	Average Group Duration			Difference from Control Group			
				MIA[1] vs. Control Group		MIA[2] vs. Control Group	
	MIA[1] Mean (SD)	MIA[2] Mean (SD)	Control Group Mean (SD)	Estimate (SE)	*p* Value	Estimate (SE)	*p* Value
Startbox [a]	19.7 (11.7)	21.7 (12.2)	17.1 (9.7)	5.4 (5.9)	.37	5.8 (5.3)	.29
Social Arm [a]	51.1 (15.4)	50.7 (10.4)	48.5 (21.0)	2.7 (9.7)	.78	2.3 (8.7)	.80
Nonsocial Arm	49.2 (19.7)	47.6 (8.7)	54.4 (24.5)	−8.1 (11.1)	.48	−8.1 (10.0)	.43
	Average Group Frequency			Difference from Control Group			
Coo Alone [b,c]	2.3 (3.0)	3.7 (2.9)	4.9 (2.0)	−.8 (.4)	.06	.6 (.4)	.14
Coo to Novel [c] Animal[2]	2.1 (1.7)	2.3 (1.4)	3.0 (2.2)	−.2 (.4)	.53	−.3 (.3)	.45
Total Coo [b]	4.4 (4.5)	6.0 (4.2)	7.9 (3.3)	−2.3 (1.9)	.24	−2.0 (1.8)	.28
Stereotypies [c]	2.5 (4.2)	4.2 (2.9)	.7 (.6)	.6 (.4)	.14	1.3 (.4)	.002

Average group behaviors are based on observed duration or frequency of behaviors over 2-minute trials. Differences from control groups are estimated from mixed-effects regression models fitted to the duration or frequency of behaviors and adjusted for gender. Descriptive statistics and summary (parameter estimates and standard errors) of the mixed-effects models assessing the relationship between group and duration and frequency of behavioral variables.

MIA[1], first trimester maternal immune activation; MIA[2], second trimester maternal immune activation.

[a]Analyses for these duration variables further adjusted for the day of the trial and gender of the stimulus monkey.

[b]Analyses for these frequency variables further adjusted for the gender of the stimulus monkey.

[c]Frequency variables square-root transformed to improve their normality.

Table 5.

Duration and Frequency of Behaviors in Juvenile Social Approach Paradigm

Behavior	Average Group Duration			Difference from Control Group			
				MIA[1] vs. Control Group		MIA[2] vs. Control Group	
	MIA[1] Mean (SD)	MIA[2] Mean (SD)	Control Group Mean (SD)	Estimate (SE)	*p* Value	Estimate (SE)	*p* Value
Proximity/Contact to Empty Cage[a]	67.7 (29.1)	71.1 (42.8)	39.1 (24.3)	2.9 (1.2)	.02	2.6 (1.0)	.02
Proximity/Contact to Subject Cage	207.7 (23.9)	101.0 (47.1)	109.9 (85.9)	96.3 (36.8)	.02	−9.6 (33.0)	.77
Social Chamber	427.2 (48.9)	379.8 (75.3)	425.2 (75.0)	.9 (39.2)	.98	−45.9 (35.2)	.21
Nonsocial Chamber	172.8 (48.9)	220.2 (75.3)	174.8 (75.0)	−.9 (39.2)	.98	45.9 (35.2)	.21
	Average Group Frequency			Difference from Control Group			
Coo	9.3 (11.4)	22.4 (12.9)	27.4 (12.0)	−15.9 (6.7)	.03	−4.0 (6.0)	.51
Approach	15.5 (3.4)	10.1 (5.3)	9.3 (3.6)	4.9 (2.2)	.04	.2 (2.0)	.91
Contact	14.7 (3.4)	9.5 (6.1)	8.8 (4.5)	4.6 (2.6)	.09	.1 (2.3)	.95
Proximity	7.4 (2.1)	2.3 (2.0)	2.0 (1.3)	5.1 (.9)	<.001	.2 (.8)	.84

Average group behaviors are based on observed duration or frequency of behaviors over 10-minute trials. Differences from control groups are estimated from mixed-effects regression models fitted to the duration of behaviors and adjusted for gender. Descriptive statistics and summary (parameter estimates and standard errors) of the mixed-effects models assessing the relationship between group and duration or frequency of behavior variables.

MIA[1], first trimester maternal immune activation; MIA[2], second trimester maternal immune activation.

[a]Variable square-root transformed to improve its normality.

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