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Maternal immune activation during pregnancy alters postnatal brain growth and cognitive development in nonhuman primate offspring

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42

ABSTRACT

Human epidemiologic studies implicate exposure to infection during gestation in the etiology of neurodevelopmental disorders. Animal models of maternal immune activation (MIA) have identified the maternal immune response as the critical link between maternal infection and aberrant offspring brain and behavior development. Here we evaluate neurodevelopment of male rhesus monkeys (*Macaca mulatta*) born to MIA-treated dams (*n*=14) injected with a modified form of the viral mimic, Polyinosinic:polycytidylic acid (Poly IC) at the end of the first trimester. Control dams received saline injections at the same gestational time points (*n*=10) or were untreated (*n*=4). MIA-treated dams exhibited a strong immune response as indexed by transient increases in sickness behavior, temperature, and inflammatory cytokines. Although offspring born to control or MIA-treated dams did not differ on measures of physical growth and early developmental milestones, the MIA-treated animals exhibited subtle changes in cognitive development and deviated from species-typical brain growth trajectories. Longitudinal magnetic resonance imaging revealed significant gray matter volume reductions in the prefrontal and frontal cortices of MIA-treated offspring at 6 months that persisted through the final time point at 45 months along with smaller frontal white matter volumes in MIA-treated animals at 36 and 45 months. These findings provide the first evidence of early postnatal changes in brain development in MIA-exposed nonhuman primates (NHPs) and establish a translationally relevant model system to explore the neurodevelopmental trajectory of risk associated with prenatal immune challenge from birth through late adolescence.

SIGNIFICANCE STATEMENT

Women exposed to infection during pregnancy have an increased risk of giving birth to a child who will later be diagnosed with a neurodevelopmental disorder. Preclinical MIA models have demonstrated that the effects of maternal infection on fetal brain development are mediated by maternal immune response. Since the majority of MIA models are carried out in rodents, the NHP provides a unique system to evaluate the MIA hypothesis in a species closely related to humans. Here we report the first longitudinal study conducted in a NHP MIA model. MIA-exposed offspring demonstrate subtle changes in cognitive development paired with marked reductions in frontal gray and white matter, further supporting the association between prenatal immune challenge and alterations in offspring neurodevelopment.

INTRODUCTION

The current COVID-19 pandemic highlights an urgent need to understand the association between maternal infection during pregnancy and the subsequent increased risk of offspring neurodevelopmental disorders (NDDs). Although the long-term effects of prenatal SARS-CoV-2 exposure are unknown, converging epidemiological data suggest that for a subset of women, infections during pregnancy are associated with an increased risk of NDDs in their offspring, including both schizophrenia (SZ) and autism spectrum disorder (ASD) (Estes and McAllister 2016, Kepinska, Iyegbe et al. 2020). The diversity of viral and bacterial pathogens associated with NDDs suggests that maternal immune response is the critical link between maternal infection and altered fetal neurodevelopment (Knuesel, Chicha et al. 2014). Moreover, the presence of inflammatory biomarkers in gestational biospecimens lend further support to the association between maternal immune activation and risk of offspring NDDs (Brown and Meyer 2018). Even in the absence of an NDD diagnosis, emerging evidence from human studies links variation in maternal cytokine levels during pregnancy with various offspring neurobehavioral outcomes, including alterations in brain growth, functional connectivity, behavioral, and cognitive development (Schepanski, Buss et al. 2018). Collectively, these studies suggest that changes in maternal cytokines during pregnancy can have long-lasting consequences, ranging from subtle differences in brain and behavioral development to severe NDDs.

The preclinical maternal immune activation (MIA) model has emerged as a powerful translational tool that allows investigators to manipulate maternal cytokine levels during gestation and systematically evaluate offspring neurodevelopmental consequences in a controlled environment. MIA models use immune activating agents, such as the viral mimic Polyinosinic:polycytidylic acid (Poly IC), to elicit an immune response during gestation. Across species, offspring of MIA-treated dams exhibit alterations in brain and behavioral development relevant to human neurodevelopmental and neuropsychiatric disease, supporting the initial interpretation of the MIA model as an animal model of ASD or SZ (Meyer and Feldon 2010, Careaga, Murai et al. 2017). As preclinical research evolves towards a hypothesis-based approach (Gordon 2019), cross-species MIA model comparisons in mice, rats, and nonhuman primates provide new opportunities to maximize the translational utility of this promising animal model and to explore neurobiological mechanisms underlying human NDDs (Kentner, Bilbo et al. 2018). Similarities in placental structure and physiology, gestational timelines, brain development, neuroanatomical organization, and behavioral complexity between humans and NHPs (Bauman and Schumann 2018, Testard, Tremblay et al. 2021) provide a unique opportunity to evaluate the foundational knowledge of the rodent MIA model in a species more closely related to humans.

Our research team developed the first Poly IC-based NHP model to explore the neurodevelopmental trajectory of risk associated with prenatal immune challenge in the rhesus monkey (*Macaca mulatta*). In our previous study, pregnant rhesus monkeys injected with a modified form of Poly IC at the end of the first or second trimester exhibited a transient but potent immune response and produced offspring that deviated from species-typical behavioral development (Bauman, Iosif et al. 2014, Machado, Whitaker et al. 2015, Rose, Careaga et al. 2017). Although *in vivo* imaging and postmortem brain tissue studies were limited in these initial NHP MIA cohorts, the emergence of atypical behaviors as the animals matured suggested that this model system may provide an opportunity to explore circuitry relevant to NDDs that may be vulnerable to prenatal immune challenge. Indeed, these small cohorts of MIA-treated NHPs demonstrated increased striatal dopamine in late adolescence as indexed by positron emission tomography (Bauman, Lesh et al. 2019), aberrant dendritic morphology in the dorsolateral prefrontal cortex (DLPFC) (Weir, Forghany et al. 2015) and region-specific alterations in gene expression (Page, Gandal et al. 2021). We have generated a larger cohort of late first trimester MIA-treated male offspring to carry out a comprehensive evaluation of brain and behavioral development from birth to late adolescence to characterize the emergence of brain and behavioral alterations in MIA-exposed NHPs. Here we present our initial findings of longitudinal structural magnetic resonance imaging (MRI) and cognitive performance data from this unique NHP MIA model.

METHODS

All experimental procedures were developed in collaboration with the veterinary, animal husbandry, and environmental enrichment staff at the California National Primate Research Center (CNPRC) and approved by the University of California, Davis Institutional Animal Care and Use Committee. All attempts were made (in terms of social housing, enriched diet, use of positive reinforcement strategies, and minimizing the duration of daily training/testing sessions) to promote normal social development and psychological well-being of the animals that participated in this research. Gestational timing, choice of species, source of immune activating agent, and subsequent magnitude of the maternal immune activation determine the impact on offspring neurodevelopment in preclinical MIA models. In accordance to recent guideline recommendations for improving the reporting of MIA model methods, we have completed the reporting table from (Kentner, Bilbo et al. 2018) and provided it as Table 1.

Animal selection

Pregnant dams were selected from the indoor, time-mated breeding colony based on age, weight, parity, and number of prior live births (Table 2). Candidate dams between 5 and 12 years old carrying a male fetus were assigned to MIA (*n*=14) and control/saline (*n*=10). Due to limited availability of male fetuses, untreated pregnant females confirmed to be carrying male fetuses were added to the control group (*n*=4). One offspring from the MIA group was euthanized at 6 months of age due to an unrelated health condition and is not included in behavior or neuroimaging datasets after 6 months of age. A second animal from the MIA-treated group was euthanized at 42 months due to an unrelated health condition and is not included in the final neuroimaging time point.

Maternal immune activation and validation

MIA induction protocols are based on our previous dosing and gestational timing experiments previously described (Rose, Feldman et al. 2003, Bauman, Iosif et al. 2014, Machado, Whitaker et al. 2015, Weir, Forghany et al. 2015, Bauman, Lesh et al. 2019). Synthetic double-stranded RNA (Polyinosinic:polycytidylic acid [Poly IC] stabilized with poly-L-lysine [Poly ICLC]) (Oncovir, Inc.; 0.25 mg/kg i.v.) or sterile saline (equivalent volume to Poly ICLC) was injected at 07:30 hours in the cephalic vein in awake animals on gestational day (GD) 43, 44, and 46 (Table 3). Health and behavior observations were conducted three times pre-treatment, 6 hours after each of the three injections, and three times post-treatment. The

checklist captured the presence or absence of any clinical or behavioral symptoms resulting from the infusions including change in appetite, watery eyes or nasal discharge, liquid stool, lethargy or labored movements, and body temperature. Prior to infusions, programmable temperature microchips (Bio Medic Temperature Systems, Seaford, DE) were implanted subcutaneously under sedation near the left and right clavicle, and a temperature wand then scanned the microchip and displayed body temperature. Temperatures were recorded just prior to Poly ICLC or saline injection, and 30 minutes, 6 hours, and 8 hours after injection. Pre- and post-treatment baseline temperatures were taken during health and behavior observations. Blood was collected from the dams on approximately GD 40 while sedated for ultrasound (pre-treatment), from awake animals on GD 44 and 46, 6 hours after Poly ICLC infusion, and on GD 51 or 52 while sedated for a recheck ultrasound (post-treatment) for cytokine analysis. Blood samples were centrifuged and the serum was removed, aliquoted into 200 uL samples, and frozen at -80°C until analysis. A longitudinal analysis on the maternal IL-6 response to Poly ICLC exposure was measured in serum using a NHP multiplexing bead immunoassay (MilliporeSigma, Burlington, MA) that was analyzed using the flow-based Luminex™ 100 suspension array system (Bio-Plex 200; Bio-Rad Laboratories, Inc.). Hair samples were collected from the dams at GD 150 to evaluate potential group differences in chronic stress during pregnancy.

Hair cortisol analysis

Hair samples were collected from the dams at GD 150 when they were sedated for an ultrasound by shaving the nape of the neck. Samples were then placed in tubes and stored in a -80°C freezer. Samples were not collected from three of the untreated controls, resulting in Control (*n*=11) and MIA (*n*=14). Cortisol concentrations were analyzed using established protocols (Vandeleest, Capitanio et al. 2019) by extracting cortisol from hair using methanol and then measured using a salivary cortisol kit (Salimetrics, State College, PA, USA). Intraassay coefficient of variability (CV) was 1.6%.

Rearing conditions and husbandry

Infants were raised in individual cages with their mothers where they had visual access to other mother-infant pairs at all times. For 3 hours each day, one familiar adult male and four familiar mother-infant pairs were allowed to freely interact in a large cage (3m l x 1.8m w x 2m h) to provide enrichment and facilitate species-typical social development. The rearing groups consisted of two MIA-treated mother-infant dyads and two control mother-infant dyads. Dominance hierarchies naturally formed between the dams, and group stability was monitored throughout by trained observers. The infants were weaned from their mothers

at 6 months of age and were permanently paired with a familiar peer from their rearing group. Weanlings continued the same socialization routine through approximately 18 months of age. They were transferred to the large enclosures for 3 hours each day with the same three weanlings from their rearing group, the familiar adult male, and an adult female who was not one of the dams involved in the study. Animal rooms were maintained at 18-29°C and on a 12/12 light/dark cycle (lights on at 06:00). Subjects were fed twice daily (Lab Diet #5047, PMI Nutrition International INC, Brentwood, MO), and provided with forage scratch daily and fresh produce biweekly; they had access to water *ad libitum* along with a variety of enrichment devices.

Offspring physical growth, neurodevelopmental milestones, and early behavioral development

Measures of body growth (weight, crown-rump length, and head circumference) were collected at 1 and 3 months of age as well as at the neuroimaging time points (6, 12, 24, 36, and 45 months of age). The NHP offspring described in this paper underwent comprehensive assessments of social development that will be the focus of future publications. Here data are presented on a neurobehavioral neonatal assessment conducted at 1 week of age, home cage observations of the mother-infant dyad (0-6 months), and home cage observations with their age/sex/treatment matched cage mate (6-18 months) (Table 4). All behavioral observations were carried out by trained observers demonstrating an inter-observer reliability > 85% (agreements/ [agreements + disagreements] X 100). Each infant received a dye mark, allowing the observers to record behaviors while remaining blind to their experimental condition. Detailed methods are provided in previous publications (Bauman, Lavenex et al. 2004, Bauman, Lavenex et al. 2004, Bauman, Iosif et al. 2013, Bauman, Iosif et al. 2014).

Neonatal Assessment. The development of reflexes and basic neuromotor functioning was assessed at 7 (±1) days of age. The dam was lightly sedated with ketamine to remove the infant, and the infant was transferred to a testing room. A five-point scale of maturity was used to rate the infant on measures of visual orientation and following; reflexes including rooting, righting, placing and Moro; motor maturity including head posture, coordination of movements and prone progression; and state control (agitation and consolability). For each assessment, infants were given a score of 2: present/fully developed; 1.5: mostly present/developed; 1: partially present or partially developed; 0.5: slightly developed or present; or 0: absent.

Mother-infant Home Cage Observation (0-6 months). Each mother-infant dyad was observed five days per week in the home cage by a trained observer who was familiar to the animals yet blinded to the condition of the animals. Interactions were quantified using a checklist of behaviors to describe the type and frequency

of interactions between the mother and infant. Behaviors included nursing, grooming, contact, maternal behaviors (restrain, retrieve, rejection, aggression), facial expressions, environmental exploration, and stereotypy. Observations were conducted between 08:00-15:00 and continued until weaning at approximately 6 months of age. The one-minute observation was broken into six 10-second bins, and one-zero sampling was used to record behaviors. In one-zero sampling, every behavior that is present within each bin receives a score of 1 regardless of the number of times it occurs within the bin. Behaviors that are absent within each bin receive a score of 0.

Infant-infant Home Cage Observations (6-18 months). Each infant-infant dyad was observed three days per week for 52 weeks in the home cage by a trained observer who was familiar to the animals yet blinded to the condition of the animals. Interactions were quantified using a checklist of behaviors to describe the type and frequency of interactions between familiar peers. Behaviors included sleep, nonsocial activity, proximity, contact, play, environmental exploration, and stereotypy. Observations were conducted between 08:00-15:00 beginning one week after the dyads were formed at weaning at approximately 6 months of age. The one-minute observation was scored in six 10-second bins as described above.

Cognitive Assessments

Reversal Learning (RL). Reversal learning paradigms are used across a range of species, including humans, and entail assessing cognitive flexibility in animals when reward outcomes for stimuli are reversed (Izquierdo and Jentsch 2012). Training began at 19.5 months to displace a single gray cube placed in the left, right or center position on the Wisconsin General Testing Apparatus (WGTA) within a 30-second timeframe to reveal a reward underneath. Monkeys were transported to the testing room in a modified transport box that when secured enabled them to reach out through a plexiglass panel and fully participate with the WGTA. Each daily training session consisted of 20 trials, and training continued until a criterion of 18/20 retrievals in a session. Weekly maintenance training sessions were conducted until testing began at 21 months of age. For the reversal learning assessment, monkeys were tested in 20 daily sessions using two identical cubes differing only in color (black and white). The cubes were placed to the right and left of center on the WGTA test board. The black cube was initially designated correct, and when displaced, revealed a food reward in the well underneath. The well covered by the white cube did not contain a food reward. Right/left placement of the correct cube followed a random order for the 20 trials per session. Daily testing continued until the monkeys performed 18/20 trials correct in a session. Once this criterion was reached, the reward contingencies were

reversed in the next testing session (the white cube was correct and the black cube was incorrect). When the 18/20 criterion was reached, the contingencies were again reversed. The number of reversal events monkeys were able to achieve within 20 sessions were measured. Response latency on each trial was also recorded as well as a rating of the monkey's temperament during the session. For temperament, the experimenter who tested the monkey rated its temperament shortly after the completion of the testing session. Monkeys were rated on a 0-3 scale on object orientation, goal directivity, irritability, activity, inhibition, impulsivity, stereotypies, attention to the task and its behavior during omission errors.

Automated Cognitive Testing. Automated cognitive testing via computerized touchscreen devices allowed more complex assessments during the older juvenile stage. The remaining cognitive tests were conducted with the Cambridge Neuropsychological Test Automated Batteries software (CANTAB, Cambridge Cognition, Cambridge, UK) on a modified desktop computer, touchscreen, and automated reward dispenser using protocols for rhesus monkeys (Weed, Taffe et al. 1999, Golub, Hogrefe et al. 2005, Golub, Hogrefe et al. 2014, Golub, Hackett et al. 2017). Sugar pellets (Bio-Serv, Flemington, NJ) were used for rewards. Animals were tested in their home cage and temporarily separated in place from their partner. Testing occurred between 07:30 and 11:30, and the morning meal was fed following testing. *Ad libitum* access to water was available throughout testing. Touchscreen training using successive approximation began between 32 and 33 months of age as previously described (Golub, Hackett et al. 2017). All monkeys then passed the box training module criteria of 10 consecutive box touches within 10 minutes for 2 consecutive sessions.

Continuous Performance Task (CPT). Sustained attention and response inhibition were assessed at 33-34 months of age via the CPT module on the CANTAB apparatus. CPT was designed as a go/no-go task in which three different colored boxes were presented one at a time on the screen. White boxes were correct and rewarded with a sugar pellet when touched. Red and green boxes were incorrect and were not rewarded when touched. Equal numbers of the three colored boxes were presented for 3 seconds each across 84 trials in one daily 10 minutes testing session. Correct responses were thus selecting the white box and not selecting, or inhibiting a response, to the red and green boxes. Incorrect responses were failing to select the white box or selecting the red or green boxes and resulted in a longer intertrial interval (3-second blank screen timeout). Monkeys had two attempts to touch a white box five times within 30 seconds to initiate their daily testing session and ultimately had 30 testing sessions. Performance was assessed for hits (correct responses), misses (omission errors), correct rejections (not selecting an incorrect box), false alarms (selecting an incorrect

box) and signal detection theory measures of response accuracy (d'), non-response bias, and response bias (beta).

Progressive Ratio Breakpoint Task (PRBT). Motivation with respect to reward efficacy was tested with the Progressive Ratio CANTAB task. Monkeys began testing at 40-41 months of age and had 10 daily sessions. For this task, they had to touch a blue rectangle to retrieve a food reward, and the number of touches increased geometrically every 8 trials over the testing session. For example, the monkeys had to touch the box once for a reward for the first 8 trials, but then two touches for the next 8 trials, 4 touches for the following 8 trials, 8 touches for next 8 trials, etc. Daily sessions lasted for 30 minutes or until 3 minutes passed since a box was last touched. The progressive breakpoint was considered the point at which the animal stopped responding and the session ended.

Probabilistic Reversal Learning (PRL). A more complex reversal learning paradigm was introduced at 44-45 months of age. Probabilistic reversal learning is the reversal learning test usually conducted in humans because it reduces the possibility that individuals develop a simple win-stay lose-shift strategy instead of continuing to acquire information on each trial. In this CANTAB module, two stimuli did not have a binary yes/no reward contingency as they did in the WGTA reversal learning task, but rather, one stimulus was more likely, but not guaranteed, to be rewarded instead of the other stimulus. To begin, a basic reversal learning task with 100% reward probability (one stimulus always correct and one stimulus always incorrect) was used to establish consistent performance on the computerized task. Each 30 min daily testing session consisted of 60 trials, a 30-second stimulus presentation length, 5-second intertrial interval, and 5-second darkness timeout following incorrect responses. When the monkeys reached 90% (54/60) correct in a session, the probabilistic reversal learning paradigm began the next day with two new stimuli. In this paradigm, there was a 90:10 reward ratio where one stimulus was rewarded 90% of the time and the other stimulus was rewarded 10% of the time. Thus 10% of the time, the reward contingency was possibly unexpected based on what the monkeys learned about the reward outcomes for each stimulus. When a monkey achieved 85% (51/60) correct for the session, the reward contingencies for the stimuli were reversed in the next testing session. With the same pair of stimuli, the one that was previously rewarded 90% of the time was now rewarded 10% of the time, and vice versa. As with the reversal learning paradigm in the WGTA, the number of reversal events monkeys were able to achieve within 20 sessions was assessed.

Intradimensional/Extradimensional Shift (ID/ED). The ID/ED task is a computerized adaptation of the Wisconsin Card Sorting Task and is designed to test cognitive flexibility with respect to being able to shift from

a learned set of rules to another, or attentional set shifting. Monkeys were 46-47 months old and began this task two days after completion of Probabilistic Reversal Learning. The premise of ID/ED was that the monkeys were presented with two stimuli and they learned the rule for the correct/rewarded one, and when a criterion was reached, the rule for which stimulus to select changed. There were four stages to the ID/ED task. Each stage consisted of a rewarded stimulus acquisition and then reversal of rewarded stimulus. The first stage was a Simple Discrimination (SD) in which two stimuli of different shapes were presented. One shape was rewarded until a criterion of 12 correct responses out of the last 15 trials was reached. On the subsequent trial, the previously rewarded stimulus became incorrect and the previously incorrect stimulus became correct (Simple Discrimination Reversal, SDR). When the criterion (12/15) was reached in the reversal round, the task moved to the second stage. This was the Compound Discrimination (CD) stage, in which the same shape stimuli from stage 1 were used and had the same reward contingencies as the reversal round, but the stimuli now had lines superimposed on them to create compound stimuli. The monkeys had to learn to select the previously rewarded shape no matter which of the two lines was superimposed on it in each trial. As with stage 1, the rewarded stimulus was then reversed after the monkey reached criterion (Compound Discrimination Reversal, CDR). Following criterion on stage 2, the task moved to stage 3, the Intradimensional Shift (ID). In this stage, two new shape and line stimuli were presented, yet the same relevant dimension of shape was rewarded. The monkeys had to learn both the dimension and stimulus to select for a reward. Once criterion was reached, the other shape stimulus was rewarded for the reversal round (Intradimensional Shift Reversal, IDR). After criterion was reached for both rounds of the third stage, the fourth stage was the Extradimensional Shift (ED). Two new shape and line stimuli were presented but now it was the line stimuli that were relevant for reward instead of the shapes. Once monkeys learned which dimension and stimulus was rewarded and reached criterion, the previously incorrect stimulus in the same dimension was now the rewarded stimulus and vice versa (Extradimensional Shift Reversal, EDR). Monkeys were tested in 60-minute daily testing sessions. In that timeframe, there was no limit to how many trials, reversals, and stages they went through, although performance did not carry over between sessions for contribution to criterion. On subsequent testing days, the new session began on the stage last presented in the previous session. For each trial, monkeys had 30 seconds to make a stimulus choice, a 3-second intertrial interval, and a 5-second blank screen timeout following an incorrect response. Performance on each stage was assessed by the number of trials needed to move to the next stage, the number of correct/incorrect responses and misses/omission errors. The number of misses, hits and errors were also normalized to the number of trials each monkey underwent for each stage as this was variable based on performance.

Neuroimaging

Magnetic resonance imaging was performed at approximately 6, 12, 24, 36, and 45 months of age using a Siemens Magnetom Skyra 3-T (Davis, California) with an 8-channel coil optimized for monkey brain scanning (RapidMR, Columbus, Ohio). Twenty-four of the animals were also scanned at 1 month of age. However, due to the low gray matter/white matter contrast in the T1 images, this time point is not included in the analyses for the current paper. Three animals were scanned at 3 months of age. However, some respiratory difficulties were encountered at this age and scans were discontinued in order to not put the animals at risk. It was determined that three of the animals were sensitive to isoflurane, therefore, at subsequent time points, these animals were scanned using propofol as the anesthetic. The rate of infusion varied in order to maintain the animal at a steady state of anesthesia. All other animals at all time points were sedated with ketamine for tracheal intubation, then anesthetized with isoflurane for positioning in an MR-compatible stereotaxic apparatus. Once the animal was placed in and centered at the mid-line of the stereotaxic apparatus, the 8-channel receiver coil was attached to the stereotaxic apparatus using a custom connector. The center point of the 8-channel coil was positioned at AP+10 on the stereotaxic apparatus. The Skyra table was "landmarked" at AP+10 so that the center of the animal's brain was at the isocenter of the MRI magnet. Anesthesia was maintained with isoflurane at 1.3-2.0%. Fluids were maintained with a saline infusion at a rate of 10 mL/kg/hr for the duration of the MRI scan.

Acquisition Parameters. T1 weighted images (480 sagittal slices) were acquired with TR=2500 ms, TE=3.65 ms, flip angle=7°, field of view 256 x 256, voxel size during acquisition 0.6 x 0.6 x 0.6 mm. Acquired images were interpolated during image reconstruction to 512 x 512 voxels with a final resolution of 0.3 x 0.3 x 0.3 mm. The structural imaging protocol was followed with additional sequences that will not be described in this paper.

Image Processing. All images were processed by operators unaware of group assignment. T1 weighted images were aligned into common space (Shi, Budin et al. 2016), bias field corrected, and brain masked using AutoSeg_3.3.2 (Wang, Vachet et al. 2014). Brain masks were manually corrected if necessary. Following this preprocessing, T1 weighted images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using NeosegPipeline_v1.0.8 (Cherel, Budin et al. 2015) (Figure 1). Probabilistic tissue maps from structural multi-atlas templates were applied to each subject's T1 weighted images via deformable registration. University of North Carolina lobar parcellation was employed to parcellate the tissue

segmentations into 24 lobar brain regions using the multi-atlas fusion in AutoSeg_3.3.2 (Wang, Vachet et al. 2014). For these regions, total GM and WM volumes were extracted. Lateral ventricles volume were determined via semi-automated segmentation using the region competition deformable surface approach in ITK snap (Yushkevich, Piven et al. 2006) as applied to the probability CSF maps from the tissue segmentation (Lyall, Woolson et al. 2012). All segmentation and parcellation results were visually quality controlled. No major issues were detected for any of the results.

Regions of Interest. In order to limit the number of comparisons, the regions of interest selected were based on a review of literature documenting brain alterations associated with MIA: prefrontal, frontal, cingulate, and temporal limbic (including amygdala and hippocampus) regions (Figure 2), and lateral ventricles (Piontkewitz, Arad et al. 2011, Willette, Lubach et al. 2011, Crum, Sawiak et al. 2017, Drazanova, Ruda-Kucerova et al. 2018).

Statistical analysis

Statistical analyses were conducted within a generalized linear mixed-effects models framework (McCulloch, Searle et al. 2008) that can accommodate traditional general linear models (e.g., ANOVA and multiple linear regression) for data that were assumed normally distributed and independent across individuals, as well as linear mixed-effects models for normally distributed, binary, or count data that were collected repeatedly for an individual (across time or conditions). This flexible approach allows the use of all available data for an individual and provides the ability to control for the effect of covariates of interest and to account for the intrinsic complexity of the data by modeling subject-specific random effects and residual correlations. Transformations were employed if assumptions of the linear models were not met and nonparametric techniques (exact Wilcoxon rank-sum test) were used to compare groups when transformations were unsuccessful. Logistic models were also used for event outcomes of trials. All models were validated both graphically and analytically. All tests were two-sided, with $\alpha = 0.05$. All analyses were conducted in SAS version 9.4. (SAS Institute Inc., Cary, NC).

Offspring Development Analyses. Developmental trajectories for weight, crown-rump, and head circumference were analyzed using linear mixed-effects models with fixed effects for group (MIA, control), age at measurement, and the interaction between age and group. Linear, quadratic, and cubic age effects were considered. To account for the within-animal dependence, random intercepts and slopes for linear and quadratic effect of age were included in the models.

Cognitive Development Analyses. Cognitive developmental outcomes were analyzed using repeated measures techniques: linear mixed-effects models for continuous outcomes (after square root transforming the data to meet distributional assumptions) and logistic mixed-effects models (for events observed across trials, such as misses, correct choices, false alarms). To account for the within-animal dependence, random intercepts were included in the models. For Continuous Performance Task we modeled the number of misses and false alarms and the probability to commit a miss or a false alarm (using logistic models) across the trials in each session. Models included fixed effects for group (MIA, control), session (linear, quadratic, and cubic session effects), and the interaction between session and group. Interaction terms were not significant and were not retained in the reported models. For Intradimensional/Extradimensional Shift we modeled the miss and correct rates and the probability of a miss or correct choice (using logistic models) across the trials in each stage. Models included fixed effects for group (MIA, control), stage (categorical 8 stages), and their interaction. The interaction terms were removed if they were not significant. For reversal learning tasks, nonparametric statistics were used to examine group differences in performance during individual sessions following the first reversal.

MRI Analyses. The primary aim of the MRI analyses was to model brain growth from 6 to 45 months and to assess whether MIA-treated animals had a different developmental trajectory than the control animals. Separate models were fitted for each of the global measures (brain volume, GM, WM, L. Ventricles). We first fitted models with fixed effects for group (MIA, control), time (6, 12, 24, 36, or 45 months), and the interaction between group and time, using an exchangeable within-animal covariance (except for L. Ventricles, for which a spatial exponential covariance was used) to account for the correlated nature of the data due to the repeated measures. If the interaction did not add significantly to the model, it was removed, and the results of the model including only main effects were reported. For region of interest (ROI) analyses, two sets of models were fitted. The first set of models paralleled the global measures analyses; the second set included adjustment for the total brain volume. Separate models were fitted for GM and WM bilateral volumes in frontal, prefrontal, cingulate, and temporal limbic cortices. The core models included fixed effects for group (MIA, control), time (6, 12, 24, 36, or 45 months), interaction between group and time, and total brain volume (for the adjusted models). Within-animal dependence was modeled using an unstructured covariance structure. We removed the interaction from the reported models if it was not significant. Significant interactions between group and time were followed up by tests to evaluate time-specific group differences.

Maternal Cytokines and Offspring Neurodevelopment. A comprehensive evaluation of the maternal cytokine response and offspring development (i.e., behavior, multimodal neuroimaging, immune development) will be the focus of future publications. Here we present an exploratory evaluation of the relationship between maternal IL-6 and volumetric brain growth summaries of MIA-treated offspring. Spearman's rank correlations examined the associations between maternal IL6 response and frontal and prefrontal GM and WM brain volumes in the offspring after reducing the serial data for each individual to summaries reflecting relevant and interpretable aspects of the data. Maternal IL-6 response was summarized by the peak level (i.e., the maximum) after second and third injections, which may be interpreted as a maximum effect of the injection. Because the imaging was performed at unequal time intervals and there was small variation in the ages at scan across offspring, each monkey's GM ROI trajectory was summarized by calculating the area under the curve, standardized by the length in the study. These correlations were conducted separately in the two groups, using only the monkeys who had complete data (12 MIA, 10 Control).

RESULTS

Validation of maternal immune activation

Blood samples collected 6 hours after the second (GD 44) and third (GD 46) Poly ICLC injections confirmed a strong pro-inflammatory cytokine response as indexed by change in IL-6 from baseline samples (Table 5). Within the control group, the highest measurable IL-6 value (peak) was significantly positively correlated with dam age at conception (Spearman's rank correlation=0.80, p = 0.005), but not significantly correlated with weight (p = 0.11). Within the MIA group, there was no significant correlation between peak IL-6 levels and maternal age (p = 0.31) and weight (p = 0.85). Dams that received Poly ICLC injections also exhibited transient sickness behaviors, including reduced appetite and fever (Tables 6-7 and Figure 3). There was no significant difference in hair cortisol concentration between groups collected at the end of the third trimester (GD 150) (Figure 4). Notably, the hair cortisol concentrations observed in the MIA-treated dams during late gestation were similar to those observed in other multiparous and laboratory-housed pregnant rhesus macaques that were not undergoing any further experimental manipulations (Dettmer, Rosenberg et al. 2015), suggesting that the protocol used for inducing and assessing maternal immune activation was not associated with lasting changes in stress as indexed by hair cortisol.

Offspring development

There were no group differences in overall health, physical development (Table 8; Figure 5) and neuromotor-reflexes, behavioral maturation, and attention (Table 9). There were also no significant group differences detected in the home cage observations with their mothers from 9-24 weeks of age (Table 10) or when observed interacting with a treatment-matched social partner in the home cage from 34-78 weeks of age (Table 11).

Cognitive Development

Reversal Learning. Overall performance was similar between groups as all monkeys achieved at least 1 reversal; however, the number of monkeys meeting criterion on subsequent reversals diminished steadily in a non-group specific manner. Only the sessions up to and including the first reversal were analyzed as this was the only reversal all subjects achieved. There was no significant difference in the percent errors made between the two groups either before or after the first reversal as demonstrated in Figure 6a. However, following the first reversal MIA-treated offspring had a significantly higher number of omission errors (Figure 6b), a trial in which they failed to give a response within the 30-second timeframe. Temperament scores following the first reversal session rated behavior of the majority of the MIA-treated animals during omission error trials as

"apathetic/inactive" (data not shown). Temperament scores for object orientation, goal directivity, irritability, activity, inhibition, impulsivity, stereotypies and attention did not differ between groups.

Continuous Performance Task. Groups performed similarly on most CPT measures such as hit rate, number of misses (omission errors), average correct rejections, response bias (beta), signal detection theory measures of response accuracy (d'), and non-response bias (c) (Figure 7a-f). However, the MIA-treated group was significantly more likely to false alarm (Figure 7g) in a mixed-effects logistic model (odds ratio [OR] = 1.81, $p = 0.03$). The group difference in square root transformed number of false alarms did not reach statistical significance ($p = 0.08$).

Progressive Ratio Breakpoint Task and Probabilistic Reversal Learning. Performance on the PRBT was similar between groups with no differences observed on the highest ratio completed, session length, total number of responses, or total reinforcers earned between the two groups (Figure 8a-d). The groups also had similar performance on the PRL task with each group having a similar number of sessions to reach the first reversal (Figure 9a), performance after the first reversal (Figure 9b), proportion of animals completing each reversal (Figure 9d) and number of sessions to each reversal (Figure 9e). Interestingly, there was no difference in the number of omission errors following reversals in this task (Figure 9c). Similarly, win-stay lose-shift behavior did not differ between groups (data not shown).

Intradimensional Extradimensional Shift. Similar to past studies, both groups made significantly more errors during the extradimensional shift than the intradimensional shift, showing an attentional set had been formed (Baxter and Gaffan 2007, Weed, Bryant et al. 2008). No difference between groups was demonstrated by the number incorrect at each stage (Figure 10a), the number of choice trials (Figure 10c), or the error rate at each stage (Figure 10d). However, during the SDR and CDR stages the MIA-treated offspring was significantly more likely to miss (i.e., commit an omission error) in mixed-effects logistic models (SDR: OR = 5.48, $p = 0.01$; CDR: OR = 6.89, $p = 0.003$) (Figure 10b). The group difference in square root transformed miss rate remained significant in linear mixed-effects model for SDR ($p = 0.01$) and approaches statistical significance for CDR ($p = 0.051$). No significant group differences in miss rate were observed for other stages.

Neuroimaging

Table 12 summarizes the volumetric measures for the two groups and Table 13 displays the results of the linear mixed-effects linear models for global measures. The two groups had parallel growth trajectories from 6 to 45 months on all global measures, i.e. total brain volume, total gray and total white matter volume, and lateral ventricle volume; none of the group-by-time interactions reached statistical significance. Total brain volume from 6 to 45 months for the MIA-treated animals was consistently smaller than for the control animals, although the difference was not significant (estimated difference [est.] = -5654 mm^3, p = 0.12). The same pattern of smaller volume in the MIA-treated animals relative to controls was present in total gray matter (est. = -3762 mm^3, p = 0.12).

Table 14 summarizes the results of the unadjusted analyses for gray and white matter volumes in the four regions of interest. For gray matter, the two groups had parallel developmental trajectories from 6 to 45 months in all four regions; none of the group-by-time interactions reached significance. Yet, for the gray matter in the frontal and prefrontal regions (Figure 11a-b), MIA-treated monkeys had smaller volumes than controls did at 6 months, and these differences persisted at later ages (Frontal: est. = -564.6 mm^3, p = 0.005, Prefrontal: est. = -695.8 mm^3, p = 0.04). Table 15 summarizes the results of the analyses after adjusting for total brain volume. The magnitude of the group differences decreased, but remained significant, in both frontal and prefrontal gray matter (Frontal: est. = -403.3 mm^3, p = 0.01, Prefrontal: est. = -387.6 mm^3, p = 0.02).

For frontal white matter, there was an interaction between time and group in both unadjusted and adjusted analyses. The two groups had similar levels of frontal white matter at 6 months, but had significantly different growth trajectories over time (Figure 11c), resulting in the MIA group having lower volumes by 36 months (est. = -353.7 mm^3, p = 0.047) and more pronounced differences at 45 months (est. = -436.3 mm^3, p = 0.02). These differences persisted after adjusting for total brain volume, although the magnitude of the group differences decreased (36 months: est. = -187.0 mm^3, p = 0.050, 45 months: est. = -264.1 mm^3, p = 0.01). Furthermore, the growth of frontal white matter volume from 6 months to 24, 36, and 45 months was significantly smaller in the MIA group relative to the Control group (Table 14). A similar effect was seen when adjusting for total brain volume, with frontal white matter volume growth significantly smaller from 6 months to 12, 36, and 45 months in the MIA group relative to the Control group (Table 15).

Maternal Cytokines and Offspring Neurodevelopment

The correlation analysis for MIA treated animals revealed a consistent pattern of negative association between peak maternal IL-6 response and summary measures of both GM (Spearman's ρ = -0.50, p = 0.10 in

prefrontal; ρ = -0.55, p = 0.06 in frontal) and WM (ρ = -0.63, p = 0.03 in prefrontal; ρ = -0.62, p = 0.03 in frontal) in prefrontal and frontal regions.

DISCUSSION

Here we present initial results from a new cohort of male MIA-treated NHP offspring undergoing a comprehensive assessment of brain and behavioral development from birth through 4 years of age, a time frame spanning early infancy through late adolescence. We have previously demonstrated that rhesus monkeys exposed to prenatal immune challenge develop aberrant behaviors after a period of early typical development (Bauman, Iosif et al. 2014, Machado, Whitaker et al. 2015, Rose, Careaga et al. 2017). Although evidence of increased striatal dopamine was detected in late adolescence (Bauman, Lesh et al. 2019), other aspects of neurodevelopment have not been explored in the NHP MIA model. Here we describe striking reductions in frontal lobe volume throughout development paired with the emergence of subtle changes in cognitive performance detected in the first evaluation of cognitive development in a NHP MIA model. These findings demonstrate the translational utility of the NHP model to evaluate the emergence of neurodevelopmental changes in a species more closely related to humans and to highlight ongoing developmental changes in the anatomy of the frontal lobes as a potential marker of MIA-induced risk.

Compared with controls, the MIA-treated offspring exhibited reductions in frontal and prefrontal gray matter volumes at 6, 12, 24, 36, or 45 months and smaller increases in frontal white matter resulting in significantly reduced white matter volumes at the latter time points. Volumetric reductions have emerged as a consistent outcome in rodent MIA models (for review, (Guma, Plitman et al. 2019) and have also been reported following prenatal influenza exposure in NHPs (Short, Lubach et al. 2010). Although comparing developmental trajectories across species is challenging, it is noteworthy that reduced frontal volume has been detected in both mid-gestation MIA-treated rats (Piontkewitz, Arad et al. 2012, Crum, Sawiak et al. 2017) and late first trimester MIA-treated NHPs. Delay in frontal white matter growth volume emerging in MIA-treated offspring between 2-3 years of age observed in this study indicate a deviation from the species-typical increased white matter volumes seen from birth through puberty (Malkova, Heuer et al. 2006, Knickmeyer, Styner et al. 2010). These findings highlight the frontal lobe as a particularly vulnerable region to prenatal immune challenge in NHPs, which is consistent with our preliminary findings of subtle changes in DLPFC dendritic morphology (Weir, Forghany et al. 2015). Indeed, rodent MIA models have also identified numerous changes in neuronal migration, number, density, and alterations in dendritic structure and synapse formation that could contribute to aberrant brain growth trajectories (for review, (Bergdolt and Dunaevsky 2018)). In the present study, dams received Poly ICLC injections on GD 43, 44, and 46, which corresponds to late first trimester (GD 0-55) of the 165-day gestation. In rhesus monkeys, first trimester peak neurogenesis of

subcortical structures is followed by the early stages of corticogenesis that continues through the second trimester (GD 56-110) (Rakic 1988). Emerging evidence indicate that microglia play a critical role in regulating cell production during this time and raises the possibility that MIA-induced changes in the maternal-fetal immune environment could alter the timing and trajectory of these critical neurodevelopmental processes (Barger, Keiter et al. 2019).

Despite the significant reduction in gray matter and white matter volumes observed in the MIA-treated animals as described above, the MIA-treated animals had similar overall cognitive performance to control groups with some subtle differences. The MIA-treated offspring showed evidence for increased omission errors in the reversal learning and more misses during two stages of the ID/ED (both reversal stages), which are measures of the subject not providing a response. These behavioral changes may reflect difficulty adaptively forming and using a task set but could also reflect an increased impact of unexpected negative feedback on MIA performance. Given that MIA-treated animals performed similarly to controls on all endpoints of PRBT, motivation does not appear to be a major contributing factor. Additionally, the MIA offspring also had a significantly increased number of false alarms on the CPT. When tested with a probabilistic RL paradigm at 4 years of age, no differences were found in overall performance or number of omission errors observed. The different outcomes in omission errors in the reversal learning task versus the PRL could be a factor of maturity or a result of the different testing parameters. In the PRL task the monkey would have an expected slowed rate of learning requiring a more complex strategy to achieve a reward as compared to the RL task, and the increased complexity could have resulted in improved task engagement (Izquierdo and Jentsch 2012). In both the RL and ID/ED task, when the MIA offspring were engaged and participating, their performance was similar to the control group.

Overall, it appears the MIA offspring performed similarly to controls but had occasional trouble maintaining and using the rule associated with the tasks. It is important to note that the NHPs in the current study were evaluated from infancy through late adolescence, when cognitive performance strategies continue to mature (Weed, Taffe et al. 1999). It is plausible that the subtle changes in early cognitive performance observed in these young MIA-treated monkeys may represent a “prodromal” phase in the model that may become more pronounced over time as the monkeys progress from adolescence to young adulthood. Indeed, though many studies utilizing an adult rodent MIA model have described impairments in working and spatial memory (Bergdolt and Dunaevsky 2018, Haddad, Patel et al. 2020), studies testing juvenile age groups have produced mixed results with some reporting various deficits in cognition during prepubescent time points

(Vuillermot, Joodmardi et al. 2012, Giovanoli, Notter et al. 2015), whereas others found deficits that only emerged during adulthood (Meyer, Schwendener et al. 2006, Richetto, Calabrese et al. 2014).

Our NHP Poly IC-based MIA model is designed to stimulate inflammatory cytokine response late in the first trimester that mimics a moderate to severe infection during pregnancy. The MIA-treated dams exhibit transient fever, reduced appetite, and elevated inflammatory cytokines, including IL-6, which is necessary and sufficient for MIA to alter brain development and behavior in rodent offspring (Smith, Li et al. 2007) and has recently been associated with neurodevelopmental outcomes in monkeys (Ramirez, Graham et al. 2020) and humans (Rasmussen, Graham et al. 2021). Although the MIA model has historically been presented as a model relevant for ASD and/or SZ, emerging consensus in the field suggests that prenatal immune challenge may serve as a "disease primer" that in combination with other genetic or environmental factors may result in altered brain and behavior trajectories relevant to a number of neurodevelopmental disorders (Meyer 2019). While comparisons between animal models and clinical disorders must be made with caution, the NHP model more closely approximates the protracted period of postnatal development needed to evaluate the emergence of MIA-induced neurodevelopmental changes. The subtle impairments of cognitive performance exhibited by the MIA-treated animals does align with the premorbid phase of schizophrenia, characterized by attentional and other cognitive deficits in later childhood and adolescence (McCutcheon, Reis Marques et al. 2020) that become more severe over time (Guo, Ragland et al. 2019). The reductions in cortical gray matter volume observed in this new cohort of MIA-treated monkeys also align with neuroimaging studies from adolescents and young adults diagnosed with SZ (Schwarz, Doan et al. 2019, Keshavan, Collin et al. 2020) and may serve as a biomarker of neurodevelopmental risk (Ursini, Punzi et al. 2021).

Additional clinical and translational research is needed to understand how other factors, including genetic risk, gestational timing, nature and intensity of the maternal immune response, and additional postnatal events, determine which (if any) disease phenotype results from prenatal exposure to MIA. The vast majority of MIA models are carried out in rodents, though there is increasing interest in cross-species approaches using other species, including ferrets and pigs (Li, Dugyala et al. 2018, Rymut, Bolt et al. 2020). Although the results of the present study extend the results of rodent MIA models into a species more closely related to humans, there are inherent logistical constraints to NHP studies. Limitations of the current study include a relatively modest sample size and exclusion of female offspring. We recognize that sex differences are emerging as a critical factor in MIA model studies (Coiro and Pollak 2019) and will be focusing on the impact of MIA on the female NHP brain in our upcoming studies. As the NHP model requires 165-day gestation followed by four years of data acquisition, the complete characterization of offspring brain and

behavioral development will require additional time for coding and analysis. However, the initial observation of reduced frontal gray and white matter volume paired with subtle changes in cognitive development contributes to mounting evidence that early exposure to prenatal immune challenge triggers a pattern of divergent neurodevelopmental trajectories in MIA-treated offspring. Data collected from this unique NHP cohort will allow us next to explore behavioral, transcriptional, brain network, and immunological profiles of MIA-treated offspring to better understand resilience and susceptibility to prenatal immune activation.

Table 1. MIA model reporting guidelines.

(See PDF)

Table 2. Summary of dam characteristics.

	MIA (*n* = 14)	**Control (*n* = 10 saline, *n* = 4 untreated)**
Dam Characteristic	**Mean (*SD*)**	**Mean (*SD*)**
Age at Conception (years)	9.2 (2.4)	8.7 (2.2)
Weight at GD 40 (kg)	7.5 (1.5)	7.7 (1.6)
Prior Conceptions	4.7 (2.0)	3.6 (2.2)

Abbreviations: MIA, maternal immune activation; SD, standard deviation; GD, gestational day.

635 **Table 3.** Maternal Response to Poly ICLC.

Gestational Day (GD)	Poly ICLC Injection	Temperature	Appetite	Cytokine
Baseline (minimum 24 hours before first injection)	---	Baseline Temperature	1:30pm Three assessments across 10 days	8:00 am Baseline blood draw
GD 43	7:30am Injection #1	7:30am 8:00am 1:30pm 3:30pm	1:30pm	---
GD 44	7:30am Injection #2	7:30am 8:00am 1:30pm 3:30pm	1:30pm	1:30pm Blood drawn 6 hours after injection #2
GD 45	No injection	---	---	---
GD 46	7:30am Injection #3	7:30am 8:00am 1:30pm 3:30pm	1:30pm	1:30pm Blood drawn 6 hours after injection #3
Baseline (minimum 96 hours after last injection)	---	Baseline Temperature	1:30pm Three assessments across 10 days	8:00am Baseline blood draw

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638 **Table 4.** Home Cage Interaction Behavioral Ethogram.

Behavior	***Description***
Maternal Contact (pre-wean only)	
Breast Contact	Focal infant suckles mother.
Ventral Contact	Ventral surface of the focal contacts ventral surface of dam.
Other Contact	Other physical contact (not breast contact or ventral contact) with dam.
No Contact	No physical contact between infant and dam.
Maternal Interaction (pre-wean only)	
Maternal Restrain	Mother physically interferes with the infant's attempts to move away from her.
Maternal Retrieve	Mother physically brings infant closer to her.
Maternal Reject	Mother physically prevents the focal infant from contact.
Exploratory Events (composite score)	
Toy Play	Oral or manual manipulation of toys in cage.
Oral Explore	Oral manipulation to any part of the cage excluding food.
Manual Explore	Manual manipulation to any part of the cage.
Total Stereotypies (composite score)	
Pace	Repetitive undirected pacing with the same path repeated at least 3 consecutive times.
Head Twist	Throwing of the head back and to the side in an exaggerated manner.
Backflip	Repetitive backflip at least two times in a row.
Bounce	Repetitive bounce up and down at least two times in a row.
Nipple Clasp	Holding of the nipple.
Rock	Stationary rocking either back and forth or side to side.
Swing	Swinging within the cage for at least 3 seconds.
Self-bite	Biting motion of own limb or body part.
Salute	Fingers or hand held in place along the brow, eyes or other part of the upper face.
Other Abnormal Behavior	Any other abnormal behaviors not described above.
Infant-Infant Interaction (post-wean only)	
Non-Social Activity	Not in proximity, contact, play or other social activity.
Home cage proximity	Both animals are in the same cage.
Contact	Any physical contact between the focal animal and another.
Play	Any instance of play (contact play, wrestle play, chase).

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642 **Table 5.** Maternal IL-6 response.

	MIA (*n* = 14)		Control (*n* = 10)	
	Mean (*SD*)	**Median [Range]**	**Mean (*SD*)**	**Median [Range]**
Il-6 (pg/ml)				
Pre-dosing (GD 40)	10.9 (28.7)	0.9 [0.0 – 108.3]	0.6 (0.6)	0.4 [0.0 – 1.8]
After second injection (GD 44)	777.9 (1068.6)	450.9 [90.8 – 4341.7]	4.6 (9.6)	1.0 [0.0 – 31.4]
After third injection (GD 46)	493.0 (751.4)	130.4 [51.7 – 2780.7]	6.8 (18.6)	0.4 [0.0 – 59.5]
Il-6 change from baseline (pg/ml)				
After second injection (GD 44)	767.3 (1072.9)	450.9 [89.1 – 4341.7]	4.0 (9.4)	0.6 [-0.7 – 30.4]
After third injection (GD 46)	482.3 (754.0)	129.8 [50.0 – 2777.7]	6.2 (18.6)	0.0 [-0.2 – 59.1]

643 Abbreviations: MIA, maternal immune activation; SD, standard deviation; GD, gestation day.

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Table 6. Descriptive statistics for appetite changes during Poly ICLC injections.

	MIA (*n* = 14)		Control (*n* = 10)	
Time	**Mean (*SD*)**	**Median [Range]**	**Mean (*SD*)**	**Median [Range]**
Pre-treatment[a]	1.1 (0.7)	1.3 [0 – 2]	1.5 (0.4)	1.5 [0.7 – 2]
Treatment	0.4 (0.4)	0.3 [0 – 1.3]	1.6 (0.4)	1.7 [0.7 – 2]
Post-treatment[b]	1.3 (0.5)	1.3 [0.3 – 2]	1.3 (0.5)	1.3 [0.3 – 2]

Abbreviations: MIA, maternal immune activation; SD, standard deviation; GD, gestational day.

Pre-treatment = 3 days between GD 32 and 42; Treatment = GD 43, 44, and 46; Post-treatment = 3 days between GD 47 and 57.

Appetite was rated on a 0-2 Likert-type scale, with: 0 = *Poor (1-3 biscuits eaten)*, 1 = *Fair (4-6 biscuits eaten)*, 2 = *Good (7-9 biscuits eaten)*. For each period, scores were first summarized within-animal, by calculating the average over three days.

[a]All three days missing data for 1 animal in MIA group; [b]One day missing data for 1 animal in MIA group

Table 7. Descriptive statistics (mean, standard deviation) for temperature (^{o}C) changes during Poly ICLC (for MIA) or saline (for Control) injections.

	Gestation Day 43		Gestation Day 44		Gestation Day 46	
	MIA (*n* = 14)	**Control (*n* = 10)**	**MIA (*n* = 14)**	**Control (*n* = 10)**	**MIA (*n* = 14)**	**Control (*n* = 10)**
	Mean (*SD*)	**Mean (*SD*)**	**Mean (*SD*)**	**Mean (*SD*)**	**Mean (*SD*)**	**Mean (*SD*)**
Time-point for temperature reading						
Pre-infusion	36.6 (0.7)	36.2 (1.6)	36.7 (0.8)	36.7 (0.7)	36.6 (0.6)	36.9 (0.6)
30 minutes	36.8 (0.9)	37.0 (0.7)	36.4 (0.9)	36.9 (0.6)	36.6 (0.6)	37.1 (0.6)
6 hours	37.9 (0.6)	36.5 (0.7)	37.6 (0.8)	36.6 (0.5)	37.8 (0.8)	36.9 (0.7)
8 hours	37.3 (0.6)	36.6 (0.8)	37.3 (1.0)	36.5 (0.7)	37.0 (1.2)	36.5 (1.1)

Abbreviations: MIA, maternal immune activation; SD, standard deviation

[a]Pre-infusion temperatures were recorded immediately prior to the injection (within 1-2 minutes).

Table 9. Summary of week 1 neuro-motor reflexes, behavioral maturation and attention.

	MIA (*n* = 14)		Control (*n* = 14)		*P*-value[a]
	Mean (*SD*)	**Median [Range]**	**Mean (*SD*)**	**Median [Range]**	
Age (days)	7.0 (0.7)	7.0 [6.0 – 8.0]	6.9 (0.6)	7.0 [6.0 – 8.0]	0.95
Weight (kg)[b]	0.5 (0.1)	0.5 [0.5 – 0.7]	0.6 (0.1)	0.6 [0.4 – 0.7]	0.49
Gestation length (days)	168 (4)	167 [162 – 175]	167 (4)	167 [160 – 177]	0.97
Visual Orientation	1.3 (0.6)	1.3 [0 – 2]	1.7 (0.4)	1.8 [1 – 2]	0.14
Visual Follow	0.9 (0.4)	1.0 [0.5 – 1.5]	1.1 (0.6)	1.0 [0 – 2]	0.23
Head Posture	2.0 (0.0)	2.0 [2 – 2]	2.0 (0.0)	2.0 [2 – 2]	1.00
Coordination	0.8 (0.8)	0.8 [0 – 2]	0.7 (0.7)	0.8 [0 – 2]	0.96
Spontaneous Crawl	1.1 (0.7)	1.0 [0 – 2]	0.9 (0.7)	1.0 [0 – 2]	0.38
Rooting	0.6 (0.9)	0.0 [0 – 2]	0.4 (0.7)	0.0 [0 – 2]	0.78
Righting	1.9 (0.5)	2.0 [0 – 2]	1.9 (0.5)	2.0 [0 – 2]	1.00
Placing	1.1 (0.8)	1.0 [0 – 2]	0.8 (0.8)	0.8 [0 – 2]	0.36
Moro Reflex[c]	1.8 (0.3)	2.0 [1 – 2]	1.7 (0.4)	2.0 [1 – 2]	0.76
Predominant State	0.9 (0.5)	1.0 [0 – 2]	0.6 (0.6)	0.5 [0 – 2]	0.19
Consolability	0.5 (0.7)	0.0 [0 – 2]	0.3 (0.6)	0.0 [0 – 1.5]	0.36

Abbreviations: MIA, maternal immune activation; SD, standard deviation.

Note: All animals were rated with a score from 0 to 2 (0 = reflex absent, 0.5 = reflex slightly developed or present, 1 = partially present or partially developed, 1.5 = mostly present or developed, 2 = reflex present or fully developed). Predominant State: 0 = calm, alert, aware, 0.5 = mostly calm with slight agitation, 1 = alert but agitated for no more than half the exam, 1.5 = agitated for more than half the exam, 2 = extremely agitated throughout entire exam. Consolability: 0 = quickly consoled when picked up following exam, 0.5 = consoled after brief period of holding and swaddling, 1 = infant consoled only after prolonged holding, swaddling, rocking and/or stroking, 1.5 = brief moments of consolation and quiet after prolonged holding, 2 = inconsolable.

[a]From Wilcoxon two-sample exact tests; [b]Data missing data for 2 animals in MIA group; [c]Data missing for 1 animal in MIA group.

673 **Table 10.** Summary of pre-wean (weeks 9 to 24) home cage observations.

	MIA (*n* = 14)		Control (*n* = 14)		*P*-value
	Mean (*SD*)	**Median [Range]**	**Mean (*SD*)**	**Median [Range]**	
Breast contact	2.4 (0.8)	2.6 [1.2 – 3.6]	2.7 (0.8)	2.7 [1.5 – 3.9]	0.36
Other contact	0.7 (0.3)	0.6 [0.2 – 1.2]	0.8 (0.5)	0.7 [0.2 – 2.1]	0.96
Ventral contact	1.5 (0.9)	1.3 [0.4 – 3.8]	1.5 (0.6)	1.4 [0.5 – 2.6]	0.98
No contact	3.4 (0.9)	3.4 [1.8 – 4.8]	3.2 (0.8)	3.1 [2.0 – 4.4]	0.48
Maternal restrain	0.02 (0.04)	0.1 [0 – 0.13]	0.01 (0.02)	0 [0 – 0.07]	0.08
Maternal retrieve	0.04 (0.05)	0.02 [0 – 0.19]	0.03 (0.03)	0.03 [0 – 0.10]	0.88
Maternal reject	0.11 (0.12)	0.07 [0 – 0.44]	0.08 (0.08)	0.06 [0 – 0.31]	0.68
Total explore[a]	0.4 (0.2)	0.4 [0.1 – 0.7]	0.4 (0.1)	0.4 [0.2 – 0.6]	1.00
Total stereotypies[b]	0.003 (0.01)	0 [0 – 0.3]	0.004 (0.1)	0.0 [0 – 0.2]	0.52

674 Abbreviations: MIA, maternal immune activation; SD, standard deviation.

675 [a]Total explore includes toy play, oral explore, manual explore; [b]Total stereotypy includes pace, head twist,
676 backflip, bounce, nipple clasp, rock, swing, self-bite, salute.

677 Note: Infants were observed up to 5 times per week between 2 and 6 months. For each animal and each
678 week, behaviors were first averaged over the observations that were available (ranging from 3 to 5). All
679 animals had data between 9 and 24 weeks, so we then averaged the behaviors again within animals (from 9 to
680 24 weeks), to create a summary over the course of the study. Wilcoxon rank-sum exact tests were then fitted
681 to these averaged behaviors.

682

683 **Table 8**. Summary for the morphometric measures from 1 to 45 months.

	Age (days) *mean* (*SD*) [*Range*]		Weight (kg) *mean* (*SD*) [*Range*]		Crown Rump (cm) *mean* (*SD*) [*Range*]		Head Circumference (cm) *mean* (*SD*) [*Range*]	
	MIA (***n*** **= 14)**	Control (***n*** **= 14)**	MIA (***n*** **= 14)**	Control (***n*** **= 14)**	MIA (***n*** **= 14)**	Control (***n*** **= 14)**	MIA (***n*** **= 14)**	Control (***n*** **= 14)**
Evaluation time (months)								
1[a]	31 (2) [28-35]	32 (7) [28-54]	0.7 (0.1) [0.5-0.8]	0.7 (0.2) [0.4-1.0]	22.4 (1.2) [20.5-24.5]	21.9 (1.3) [19.3-24.0]	20.8 (0.7) [19.5-21.8]	21.1 (0.8) [19.5-22.4]
3	91 (2) [88-94]	91 (1) [88-92]	1.0 (0.1) [0.8-1.3]	1.0 (0.3) [0.6-1.7]	25.1 (1.4) [22.7-28.0]	24.7 (1.9) [20.2-28.5]	22.2 (0.9) [20.9-23.9]	22.5 (1.0) [20.2-24.1]
6	180 (2) [177-182]	180 (1) [177-181]	1.5 (0.2) [1.3-1.8]	1.5 (0.2) [1.1-1.8]	30.1 (1.8) [27.0-33.0]	30.2 (2.1) [27.0-33.5]	23.4 (0.6) [22.5-24.5]	23.9 (1.2) [21.2-26.0]
12[b]	365 (2) [364-369]	365 (1) [364-366]	2.3 (0.3) [1.9-2.9]	2.3 (0.3) [1.8-2.9]	35.8 (1.4) [34.0-38.0]	35.2 (3.1) [30.0-39.0]	24.7 (0.6) [23.8-26.0]	24.9 (0.8) [23.5-26.5]
24[b]	730 (1) [729-733]	730 (1) [729-732]	3.7 (0.5) [2.9-4.6]	3.8 (0.6) [2.8-4.7]	41.9 (2.0) [38.1-45.0]	41.9 (1.9) [38.3-44.7]	26.5 (1.0) [24.5-28.6]	27.2 (1.3) [24.5-29.5]
36[b]	1095 (1) [1093-1096]	1099 (8) [1092-1116]	5.6 (0.9) [4.5-6.9]	5.6 (1.0) [4.2-7.3]	48.6 (1.9) [45.5-51.0]	48.0 (2.9) [43.7-52.0]	28.5 (1.2) [26.4-30.2]	29.0 (1.2) [27.0-31.5]
45[c]	1372 (2) [1368-1374]	1371 (2) [1368-1374]	7.5 (1.0) [6.1-8.8]	7.4 (1.1) [5.6-8.9]	52.4 (1.7) [50.0-55.2]	52.1 (2.9) [47.0-57.0]	30.7 (1.3) [28.2-32.6]	30.6 (1.1) [28.4-32.1]

684 Abbreviations: MIA, maternal immune activation; SD, standard deviation.

685 [a]Data missing for 1 MIA and 2 Control animals for crown rump and 1 MIA and 1 Control for head circumference; [b]Data missing for 1 MIA animal on
686 all variables due to death. [c]Data missing for 2 MIA animals on all variables due to death.

687

688

Table 11. Summary of post-wean (weeks 34 to 78) home cage observations.

	MIA (*n* = 14)		Control (*n* = 14)		MIA vs. Control Estimated Difference (*SE*)	*P*-value[a]
	Mean (*SD*)	Median [Range]	Mean (*SD*)	Median [Range]		
Sleep[b]	0.03 (0.05)	0 [0 – 0.1]	0.04 (0.03)	0.04 [0 – 0.1]	-0.07 (0.05)	0.20
Non-social[b]	3.3 (0.6)	3.5 [2.2 – 4.3]	3.4 (0.6)	3.4 [2.0 – 4.1]	0.04 (0.04)	0.30
Proximity	4.0 (0.5)	4.0 [3.3 – 4.7]	3.9 (0.5)	3.8 [3.1 – 4.8]	0.07 (0.17)	0.68
Contact[b]	0.5 (0.2)	0.5 [0.1 – 0.8]	0.5 (0.2)	0.5 [0.1 – 1.1]	0.01 (0.06)	0.92
Play[b]	1.0 (0.3)	0.9 [0.5 – 1.4]	0.9 (0.3)	0.8 [0.5 – 1.4]	-0.05 (0.04)	0.31
Composite activity[b]	0.5 (0.3)	0.4 [0.2 – 1.1]	0.6 (0.3)	0.5 [0.2 – 1.1]	-0.04 (0.08)	0.67
Composite stereotypies[b]	0.2 (0.3)	0.1 [0 – 0.8]	0.2 (0.3)	0.1 [0 – 1.0]	0.02 (0.12)	0.87

Abbreviations: MIA, maternal immune activation; SD, standard deviation; SE, standard error.

[a]From linear mixed-effects models; [b]Data was square root transformed for the analysis.

Note: Infants were observed 3 times per week between 27 and 85 weeks. For each animal and each week, behaviors were first averaged over the 3 observations that were available. Not all animals were observed each week; all animals had data between 34 and 78 weeks, so we then averaged each type of behavior again within animals (from 34 to 78 weeks), to create summary behaviors over the course of the study. To these summary behaviors we fitted linear mixed-effects models with a fixed effect for group and a random effect for the "play buddy" to account for the fact that animals interacted in pairs and the exhibited behaviors were correlated.

699 **Table 12.** Summary for the gray and white matter volumetric measures (mm^3) from 6 to 45 months.

	6 Months		12 Months		24 Months		36 Months		45 Months	
	MIA	Control	MIA	Control	MIA	Control	MIA	Control	MIA	Control
	(*n* = 14)	(*n* = 14)	(*n* = 13)	(*n* = 14)	(*n* = 13)	(*n* = 14)	(*n* = 13)	(*n* = 14)	(*n* = 12)	(*n* = 14)
Age (days) at scan, *mean* (*SD*) [*Range*]										
	180 (2)	181 (5)	365 (2)	365 (1)	730 (1)	730 (1)	1095 (1)	1099 (8)	1372 (2)	1371 (2)
	[177-182]	[177-199]	[364-369]	[364-366]	[729-733]	[729-732]	[1093-1096]	[1092-1116]	[1368-1374]	[1368-1374]
Global Measures, *Mean* (*SD*)										
TBV	81429 (8113)	86200 (7724)	83522 (9692)	88781 (8550)	87821 (10381)	94268 (9060)	89446 (9242)	95287 (9372)	92406 (10297)	97857 (9671)
GM	59665 (6002)	63271 (5146)	59404 (6884)	63073 (5538)	60710 (7240)	65058 (5710)	59876 (5918)	63474 (5735)	60803 (6872)	64164 (5941)
WM	21764 (2245)	22929 (2666)	24118 (2889)	25708 (3133)	27111 (3270)	29210 (3598)	29570 (3480)	31813 (3870)	31604 (3579)	33693 (4004)
L. Ventricles	516 (186)	520 (180)	537 (248)	509 (190)	586 (344)	597 (185)	717 (367)	647 (184)	741 (407)	712 (192)
Frontal Measures, *Mean* (*SD*)										
GM	6739 (651)	7433 (521)	6853 (812)	7594 (661)	7145 (910)	8068 (736)	7220 (790)	7971 (747)	7450 (985)	8039 (800)
WM	2594 (294)	2775 (330)	2899 (347)	3152 (377)	3359 (389)	3682 (411)	3681 (428)	4035 (448)	3919 (422)	4315 (480)
Prefrontal Measures, *Mean* (*SD*)										
GM	6325 (853)	7128 (657)	6277 (949)	7078 (759)	6298 (991)	7169 (787)	6175 (819)	6942 (867)	6193 (946)	6961 (790)
WM	1586 (269)	1701 (242)	1830 (325)	2017 (284)	2151 (378)	2407 (340)	2338 (398)	2603 (364)	2479 (422)	2746 (391)
Cingulate Measures, *Mean* (*SD*)										
GM	2156 (257)	2315 (239)	2136 (271)	2337 (249)	2137 (301)	2332 (261)	2092 (247)	2269 (237)	2089 (292)	2289 (251)
WM	334 (48)	370 (59)	362 (55)	405 (66)	417 (65)	472 (79)	453 (65)	511 (81)	480 (72)	535 (82)
Temporal Limbic Measures, *Mean* (*SD*)										
GM	2416 (189)	2441 (215)	2632 (241)	2738 (241)	2928 (319)	3017 (270)	2942 (308)	3034 (316)	2989 (336)	3077 (282)
WM	432 (48)	442 (49)	463 (52)	482 (56)	533 (65)	563 (68)	564 (61)	593 (83)	596 (60)	622 (74)

700 Abbreviations: MIA, maternal immune activation; SD, standard deviation; TBV, total brain volume; GM, gray matter; WM, white matter; L.
701 Ventricles, lateral ventricles.

Table 13. Parameter estimates from the linear mixed-effects models for global volumetric measures.

	Total Brain		Gray Matter		White Matter		L. Ventricles	
Model term	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***
Intercept	86608 (2455)	<0.001	63364 (1623)	<0.001	23243 (882)	<0.001	523 (44)	<0.001
Difference (mm^3) *MIA* vs. *Control*	-5654 (3521)	0.12	-3762 (2322)	0.12	-1892 (1260)	0.15	-50 (59)	0.40
Difference (mm^3) Time 2 vs. Time 1	2363 (388)	<0.001	-247 (306)	0.42	2610 (181)	<0.001	-8 (18)	0.66
Difference (mm^3) Time 3 vs. Time 1	7278 (388)	<0.001	1411 (306)	<0.001	5867 (181)	<0.001	50 (28)	0.08
Difference (mm^3) Time 4 vs. Time 1	8589 (388)	<0.001	188 (306)	0.54	8401 (181)	<0.001	139 (34)	<0.001
Difference (mm^3) Time 5 vs. Time 1	11123 (393)	<0.001	866 (310)	0.006	10258 (184)	<0.001	187 (38)	<0.001

Abbreviations: MIA = maternal immune activation, SE = standard error, Time 1 = 6 months, Time 2 = 12 months, Time 3 = 24 months, Time 4 = 36 months, Time 5 = 45 months. Mixed-effects linear regression models were fitted to 13 MIA (1 animal is missing data at 45 Months) and 14 control animals and included fixed effects for group and time, with exchangeable within-animal covariance (except for L. Ventricles, for which spatial exponential covariance was used). One additional animal was excluded from the L. Ventricles model due to extreme data. Interactions between group and time were added to the models but were not retained in the reported models because the overall tests for time by group were not significant. The intercept can be interpreted as the predicted Time 1 volume (in mm^3) for a *Control* animal.

709 **Table 14.** Parameter estimates from the unadjusted linear mixed-effects models for regional gray and white matter volumetric measures.

	Gray Matter Volume							
Model term	**Frontal**		**Prefrontal**		**Cingulate**		**Temporal Limbic**	
	Estimate (*SE*)	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***
Intercept	7380.2 (144.0)	<0.001	7070.3 (214.4)	<0.001	2314.1 (66.8)	<0.001	2430.7 (54.6)	<0.001
Difference (mm^3) *MIA* vs. *Control*	**-564.6 (181.0)**	**0.005**	**-695.8 (319.5)**	**0.039**	-161.3 (95.6)	0.10	-4.8 (78.1)	0.95
Difference (mm^3) Time 2 vs. Time 1	129.0 (49.0)	0.01	-43.2 (40.7)	0.30	3.7 (12.3)	0.77	258.6 (19.3)	<0.001
Difference (mm^3) Time 3 vs. Time 1	515.4 (64.7)	<0.001	14.4 (49.0)	0.77	1.4 (16.6)	0.93	545.8 (27.1)	<0.001
Difference (mm^3) Time 4 vs. Time 1	501.4 (52.8)	<0.001	-162.2 (51.6)	0.004	-52.5 (13.8)	0.001	561.3 (31.0)	<0.001
Difference (mm^3) Time 5 vs. Time 1	617.2 (68.2)	<0.001	-155.0 (48.5)	0.004	-41.7 (15.8)	0.014	601.5 (27.8)	<0.001
	White Matter Volume							
Model term	**Frontal**		**Prefrontal**		**Cingulate**		**Temporal Limbic**	
	Estimate (*SE*)	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***
Intercept	2775.1 (85.0)	<0.001	1593.4 (59.5)	<0.001	350.6 (12.6)	<0.001	431.9 (11.8)	<0.001
Difference (mm^3) *MIA* vs. *Control* (at Time 1)	-187.3 (122.6)	0.14	89.8 (55.3)	0.12	2.0 (12.7)	0.88	7.5 (14.8)	0.62
Difference (mm^3) Time 2 vs. Time 1 (for Control)	377.1 (23.1)	<0.001	290.7 (15.0)	<0.001	32.7 (2.9)	<0.001	37.4 (3.2)	<0.001
Difference (mm^3) Time 3 vs. Time 1 (for Control)	907.0 (36.9)	<0.001	647.5 (25.2)	<0.001	93.6 (4.4)	<0.001	113.2 (5.0)	<0.001
Difference (mm^3) Time 4 vs. Time 1 (for Control)	1259.8 (45.5)	<0.001	838.9 (29.2)	<0.001	131.7 (5.2)	<0.001	143.6 (6.1)	<0.001
Difference (mm^3) Time 5 vs. Time 1 (for Control)	1540.1 (51.4)	<0.001	966.4 (34.5)	<0.001	154.1 (5.4)	<0.001	170.2 (5.8)	<0.001
Difference between groups in Time 2 vs. Time 1 differences	-65.7 (33.2)	0.06	-	-	-	-	-	-
Difference between groups in Time 3 vs. Time 1 differences	-135.7 (53.1)	0.02	-	-	-	-	-	-
Difference between groups in Time 4 vs. Time 1 differences	-166.4 (65.6)	0.02	-	-	-	-	-	-

Difference between groups in Time 5 vs. Time 1 differences	-248.9 (74.9)	0.003	-	-	-	-	-	-

Abbreviations: MIA = maternal immune activation, SE = standard error, Time 1 = 6 months, Time 2 = 12 months, Time 3 = 24 months, Time 4 = 36 months, Time 5 = 45 months. Mixed-effects linear regression models were fitted to 13 MIA (1 missing at 45 Months) and 14 control animals and included fixed effects for group, time and their interaction, with unstructured covariance within animal. Interactions were not retained in the reported models if the overall test for time by group was non-significant. If the interaction is not included, the estimated difference between MIA vs. Control is the same across time points, and the estimated difference between time points is the same within MIA and Control. If the interaction is included, difference between MIA vs. Control is the estimated difference at Time 1 and the estimated differences between time points are for the Control. The intercept can be interpreted as the predicted volume (in mm^3) at Time 1 for a Control animal. Statistically significant MIA vs. Control group differences ($p < .05$) bolded for emphasis.

Table 15. Parameter estimates from the linear mixed-effects models for gray and white ROI volumetric measures adjusted for total brain volume.

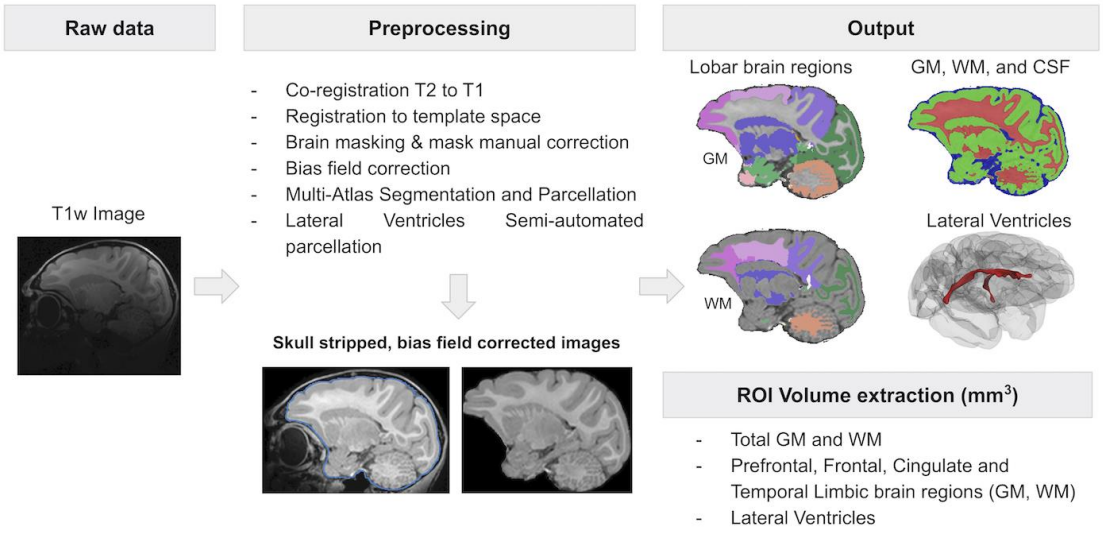
	Gray Matter Volume							
Model term	**Frontal**		**Prefrontal**		**Cingulate**		**Temporal Limbic**	
	Estimate (*SE*)	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***
Intercept	7518.4 (95.9)	<0.001	7131.8 (101.5)	<0.001	2297.9 (26.6)	<0.001	2445.6 (28.0)	<0.001
Difference (mm^3) *MIA* vs. *Control*	**-403.3 (140.9)**	**0.01**	**-387.6 (154.6)**	**0.02**	0.9 (41.1)	0.98	82.2 (44.7)	0.08
Difference (mm^3) Time 2 vs. Time 1	-91.5 (31.9)	0.007	-257.5 (23.4)	<0.001	-59.5 (10.8)	<0.001	200.7 (16.1)	<0.001
Difference (mm^3) Time 3 vs. Time 1	-163.6 (57.2)	0.006	-645.5 (51.6)	<0.001	-193.2 (19.1)	<0.001	367.5 (25.9)	<0.001
Difference (mm^3) Time 4 vs. Time 1	-299.8 (58.9)	<0.001	-941.0 (64.7)	<0.001	-282.2 (22.1)	<0.001	350.8 (33.5)	<0.001
Difference (mm^3) Time 5 vs. Time 1	-410.9 (78.3)	<0.001	-1161.8 (74.3)	<0.001	-337.6 (27.3)	<0.001	329.3 (34.2)	<0.001
Brain volume (cm^3)	93.3 (6.0)	<0.001	90.7 (5.6)	<0.001	26.7 (2.1)	<0.001	24.5 (2.4)	<0.001
	White Matter Volume							
Model term	**Frontal**		**Prefrontal**		**Cingulate**		**Temporal Limbic**	
	Estimate (*SE*)	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***
Intercept	2775.1 (42.7)	<0.001	1656.1 (33.7)	<0.001	361.5 (8.3)	<.001	441.6 (7.9)	<0.001
Difference (mm^3) *MIA* vs. *Control* (at Time 1)	-50.1 (63.4)	0.44	49.3 (44.1)	0.28	-4.4 (11.2)	0.70	9.2 (11.3)	0.42
Difference (mm^3) Time 2 vs. Time 1 (in Control)	303.4 (18.0)	<0.001	246.5 (12.0)	<0.001	24.7 (2.8)	<.001	26.6 (3.0)	<0.001
Difference (mm^3) Time 3 vs. Time 1 (in Control)	676.6 (39.7)	<0.001	511.4 (24.9)	<0.001	68.8 (5.7)	<.001	79.9 (5.3)	<0.001
Difference (mm^3) Time 4 vs. Time 1 (in Control)	1000.3 (46.3)	<0.001	678.4 (30.8)	<0.001	102.4 (6.8)	<.001	104.3 (6.6)	<0.001
Difference (mm^3) Time 5 vs. Time 1 (in Control)	1207.2 (55.9)	<0.001	758.9 (37.0)	<0.001	116.2 (8.1)	<.001	119.2 (7.4)	<0.001
Difference between groups in Time 2 vs. Time 1 differences	-52.8 (23.1)	0.03	-	-	-	-	-	-
Difference between groups in Time 3 vs. Time 1 differences	-88.9 (44.1)	0.06	-	-	-	-	-	-
Difference between groups in Time 4 vs. Time 1 differences	-136.8 (52.3)	0.02	-	-	-	-	-	-
Difference between groups in Time 5 vs. Time 1 differences	-214.0 (61.7)	0.002	-	-	-	-	-	-
Brain volume (cm^3)	28.6 (3.2)	<0.001	18.7 (2.1)	<.001	3.4 (0.6)	<.001	4.6 (0.5)	<.001

Abbreviations: MIA = maternal immune activation, SE = standard error, Time 1 = 6 months, Time 2 = 12 months, Time 3 = 24 months, Time 4 = 36 months, Time 5 = 45 months. Mixed-effects linear regression models were fitted to 13 MIA (1 missing at 45 Months) and 14 control animals and included fixed effects for group, time and their interaction, brain volume, with unstructured covariance within animal. Interactions were not

retained in the reported models if the overall test for time by group was non-significant. If the interaction is not included, the estimated difference between MIA vs. Control is the same across time points, and the estimated difference between time points is the same within MIA and Control. If the interaction is included, difference between MIA vs. Control is the estimated difference at Time 1 and the estimated differences between time points are for the Control group. Brain volume was centered at 86,200; thus, the intercept can be interpreted as the predicted volume (in mm^3) at Time 1 for a Control animal with a brain of 86,200 mm^3. The estimate for brain volume can be interpreted as the average increase in ROI volume (in mm^3) for a 1 cm^3 increase in brain volume. Statistically significant MIA vs. Control group differences ($p < .05$) bolded for emphasis.

730 **Figure Captions**

731 **Figure 1**. Structural MRI analysis workflow. MRI - magnetic resonance imaging, GM - gray matter, WM - white
732 matter, CSF - cerebrospinal fluid.
733



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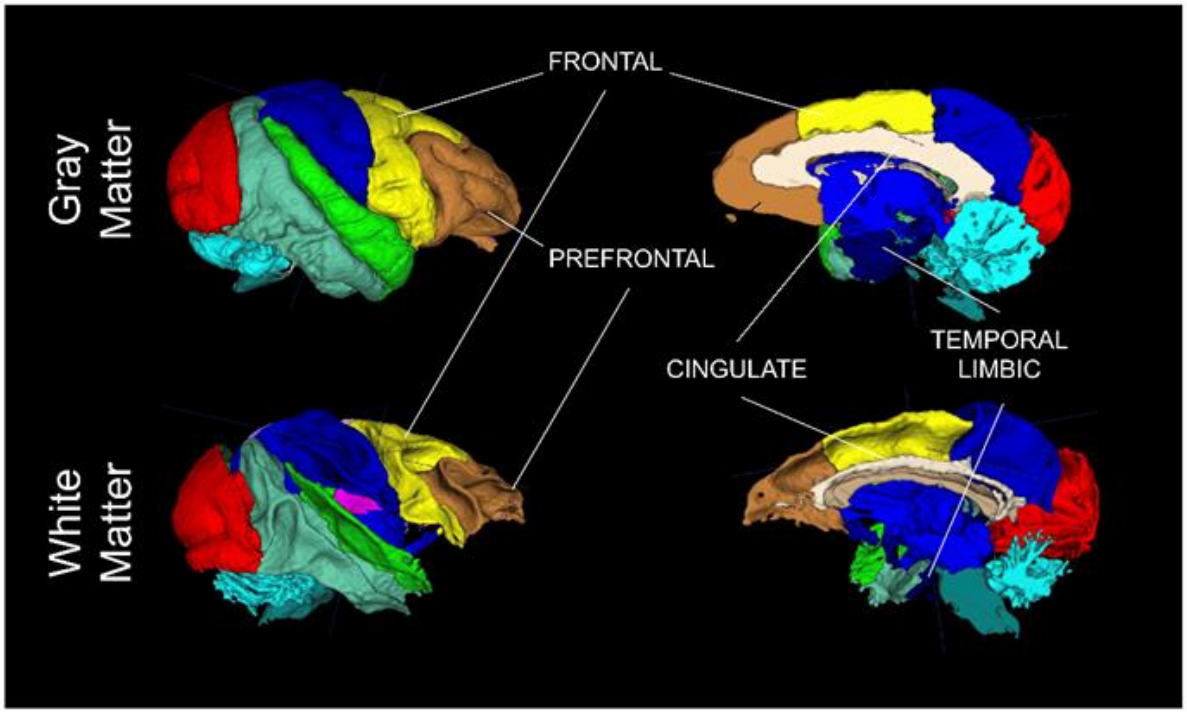
735 **Figure 2**. Regions of interest visualization.



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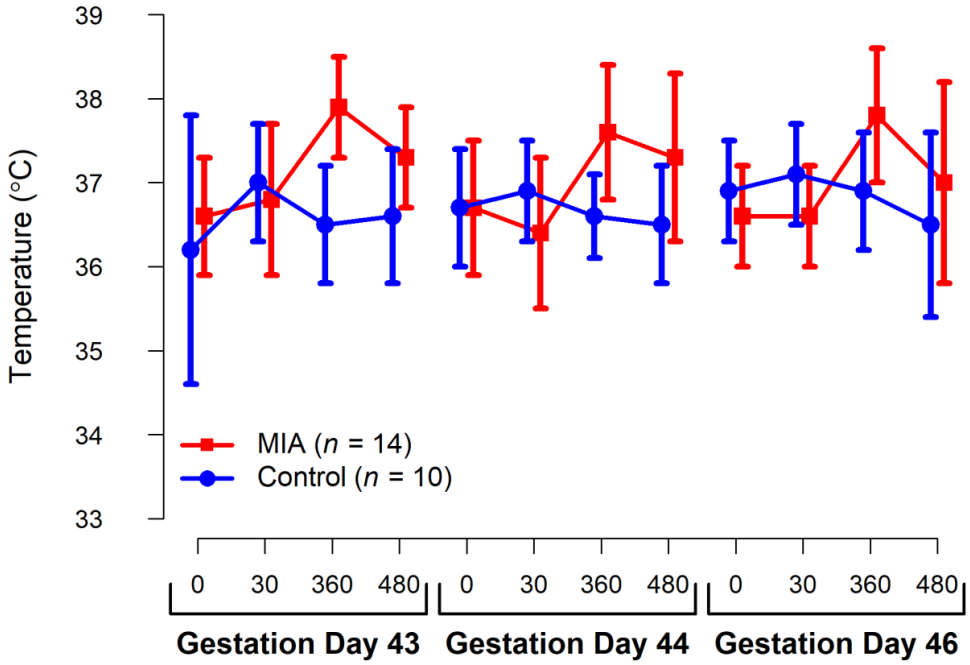
738 **Figure 3.** Average temperature for the MIA- and saline-treated dams from pre-infusion (0) through 480
739 minutes after infusion during gestational days 43, 44 and 46. Vertical bars represent 1 standard deviation.



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743 **Figure 4.** Hair cortisol concentration from MIA-exposed (*n* = 14) and Control (*n* = 11) dams. The Control group
744 includes all 10 dams that received saline injections and one untreated dam enrolled before hair sample
745 collection at gestational day 150. Horizontal lines represent group medians. Wilcoxon rank-sum exact test
746 indicated the groups did not differ (*p* = 0.32).

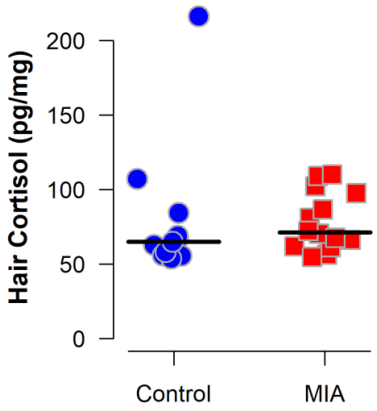

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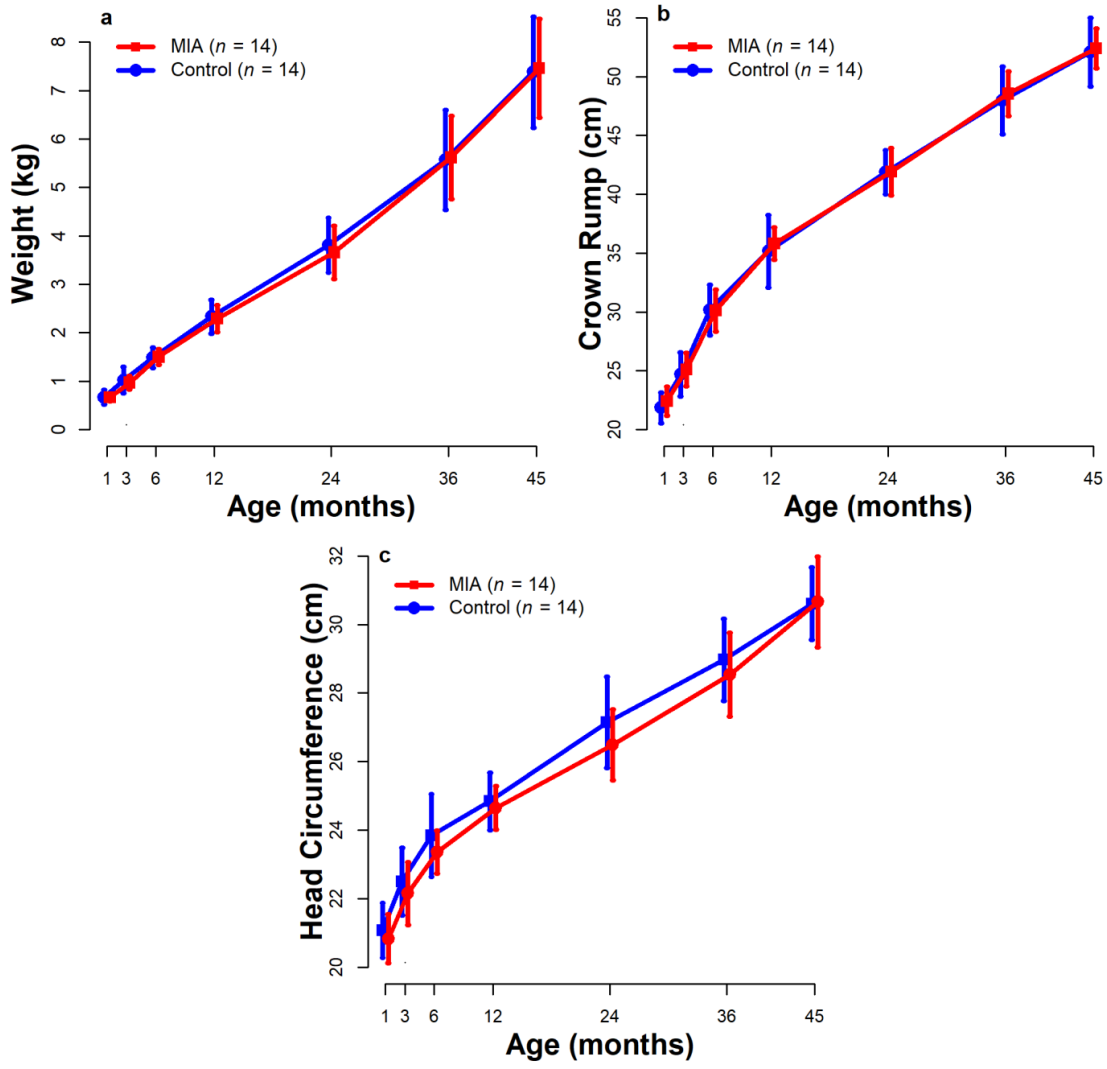
Figure 5. Average trajectory for weight (a), crown-rump length (b), and head circumference (c) for the MIA-exposed and Control offspring from 1 month through 45 months. Vertical bars represent 1 standard deviation.



Figure

Figure 6. Performance on reversal learning task of MIA-exposed (n = 13) and Control (n = 14) offspring at 21 months of age. Scatterplots show: (a) the mean error percent during the initial acquisition stage and the reversal stage and (b) the number of omission errors (i.e., non-responses) in the session following the first reversal. Horizontal lines represent group medians. **Compared to the Control group, MIA offspring have more omission errors (p = 0.005 using Wilcoxon exact rank-sum test).

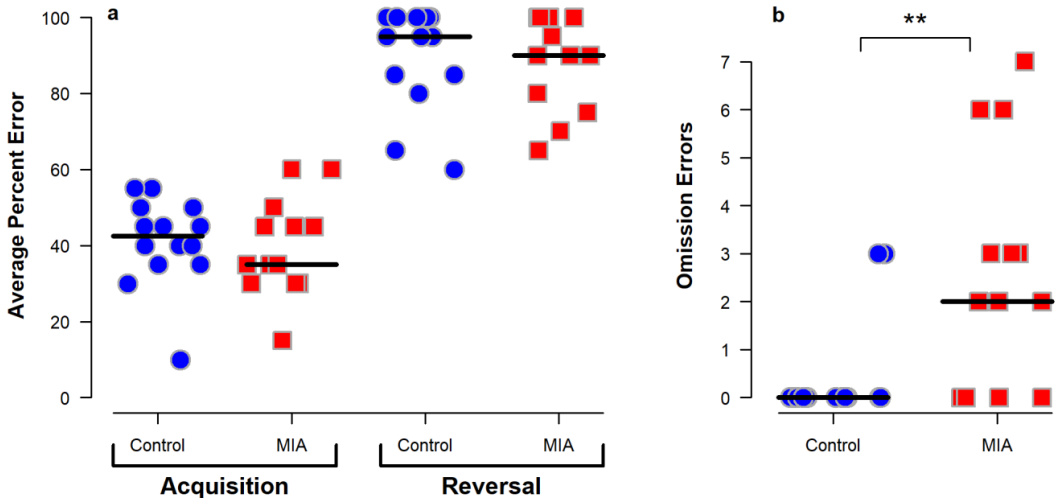


Figure 7. Continuous Performance Task performance of MIA-exposed (*n* = 13) and Control (*n* = 14) offspring at 33-34 months of age. Scatterplots show performance endpoints averaged across 20 testing sessions: (a) hit rate (correct responses), (b) misses (omission errors), (c) correct rejections (not selecting an incorrect box), (d) beta (response bias), (e) d' (response accuracy), (f) c (non-response bias), and (g) false alarms (selecting an incorrect box). Horizontal lines represent group medians. *Compared to the Control group, MIA offspring are more likely to commit false alarms ($p < .05$ using a mixed-effects logistic model).

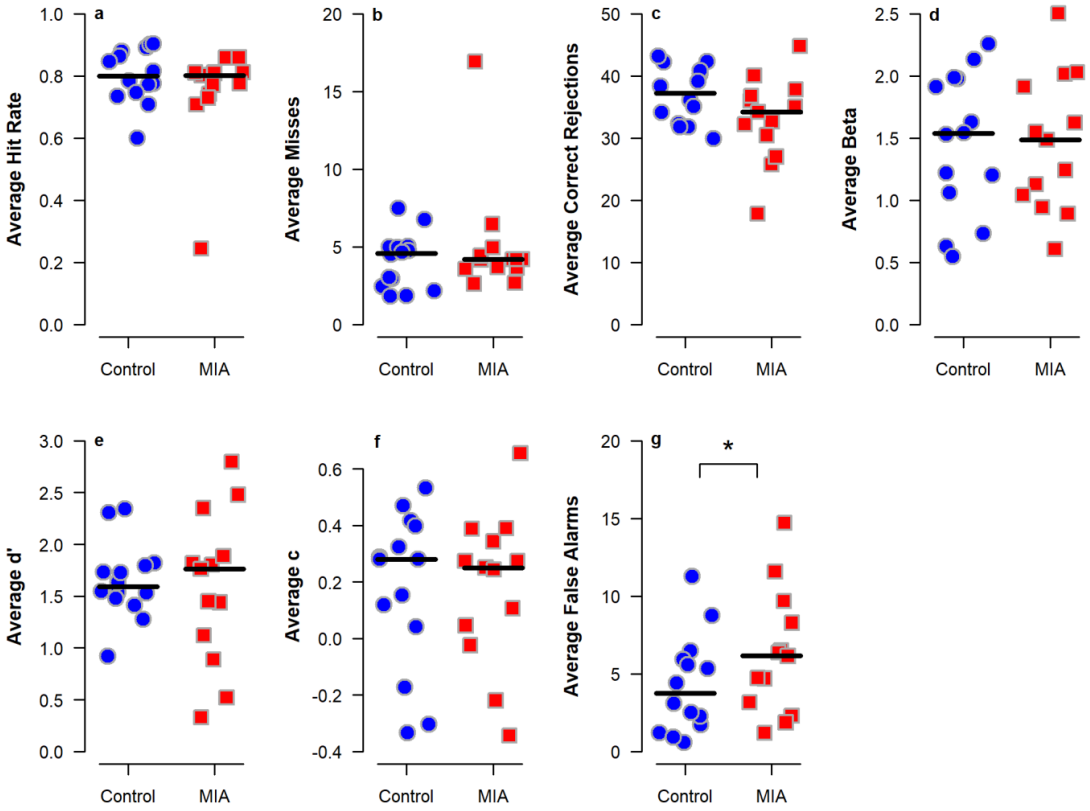


Figure 8. Progressive Ratio Breakpoint Task performance of MIA-exposed (*n* = 12) and Control (*n* = 14) offspring at 40-41 months of age. The number of screen presses required in order to obtain a food reward (the "ratio") increased geometrically over the course of the 30-minute session or until the monkey stopped responding for 3 minutes. Scatterplots show: (a) highest completed ratio, (b) duration of the test, (c) total number of responses, and (d) total reinforcers earned, averaged across the sessions for each individual. Horizontal lines represent group medians. The groups did not differ.

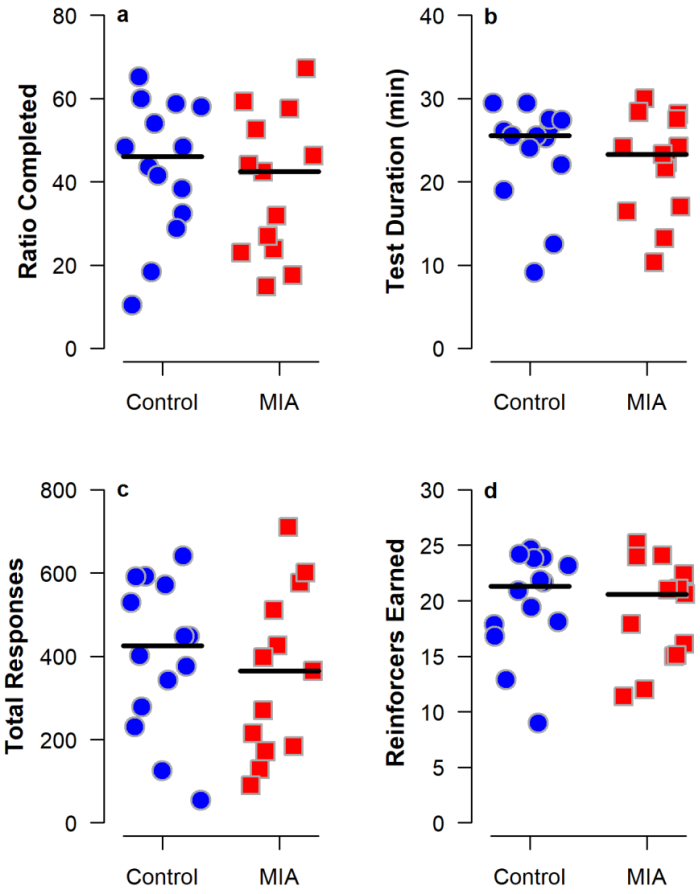


Figure 9. Probabilistic Reversal Learning task performance of MIA-exposed (*n* = 12) and Control (*n* = 14) offspring at 44-45 months of age. Plots show: (a) number of sessions required to meet criteria for the first reversal, (b) percent correct in the session following the first reversal, (c) the number of omission errors following the first reversal, (d) proportion of animals in each group achieving each reversal, and (e) the number of sessions required to meet criteria for each reversal. Horizontal lines represent group medians. The groups did not differ.

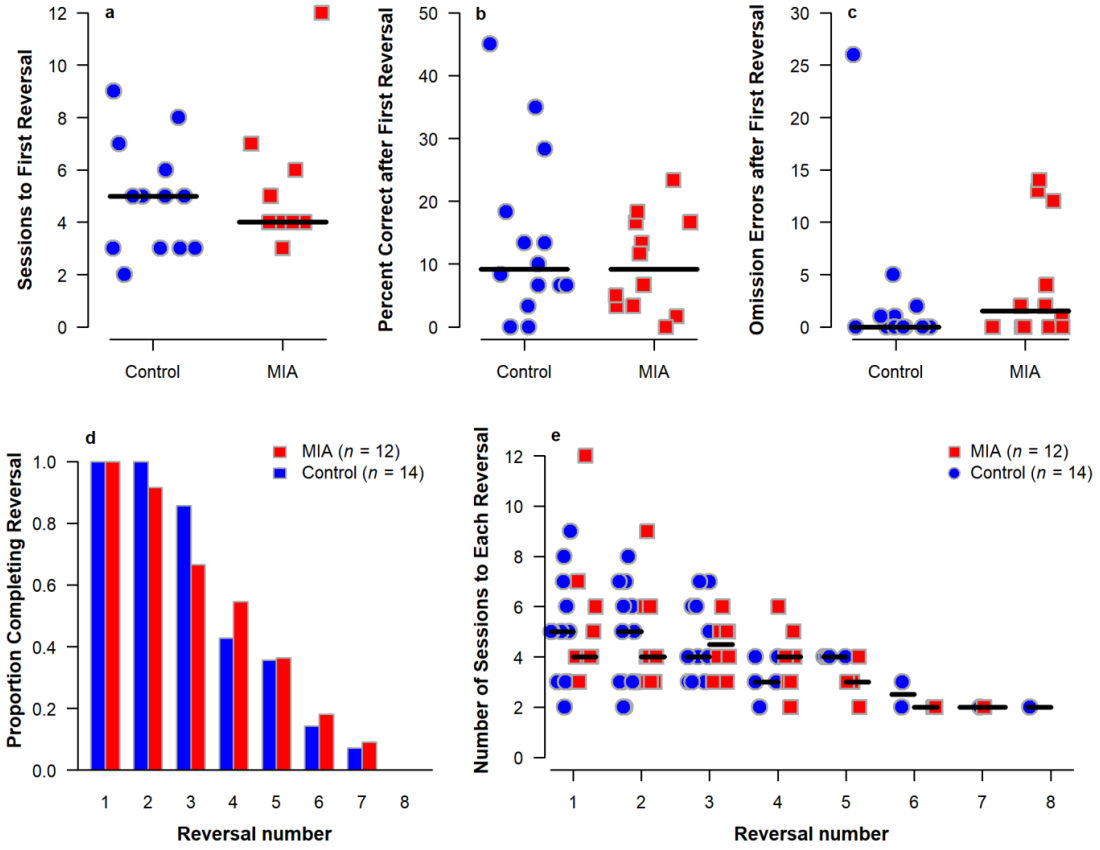


Figure 10. Performance on Intradimensional/Extradimensional Shift initiated at 46-47 months of age of MIA-exposed (n = 12) and Control (n = 14) offspring. Scatterplots show: (a) number of incorrect responses, (b) miss rate, (c) number of choice trials, and (d) error rate for each stage. Horizontal lines represent group medians. *Compared to the Control group, MIA offspring are more likely to miss during SDR and CDR (p < .05 using mixed-effects logistic models). Abbreviations: SD, simple discrimination; SDR, simple discrimination reversal; CD, compound discrimination; CDR, compound discrimination reversal; ID, intradimensional shift; IDR, intradimensional shift reversal; ED, extradimensional shift; EDR, extradimensional shift reversal.

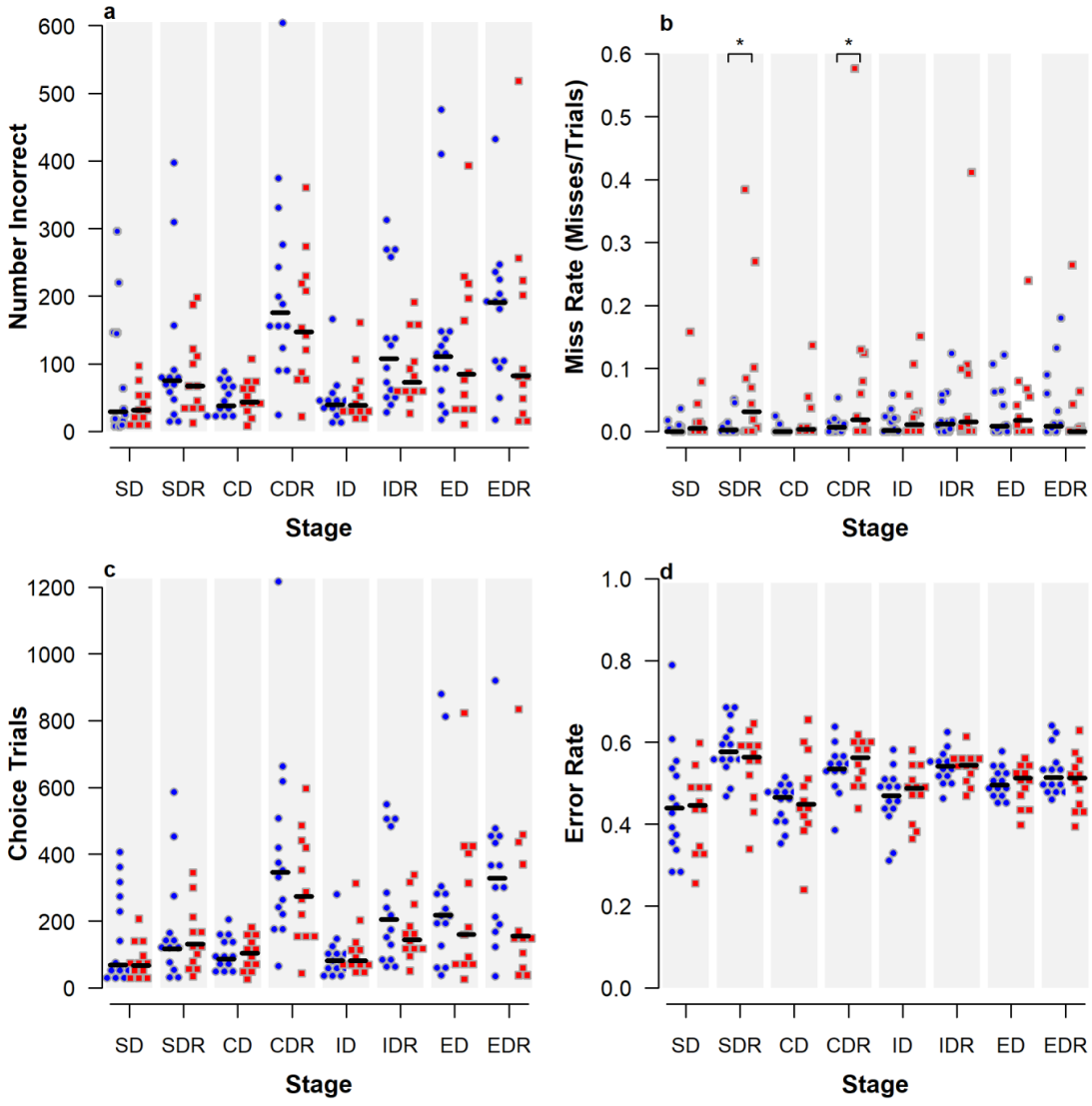
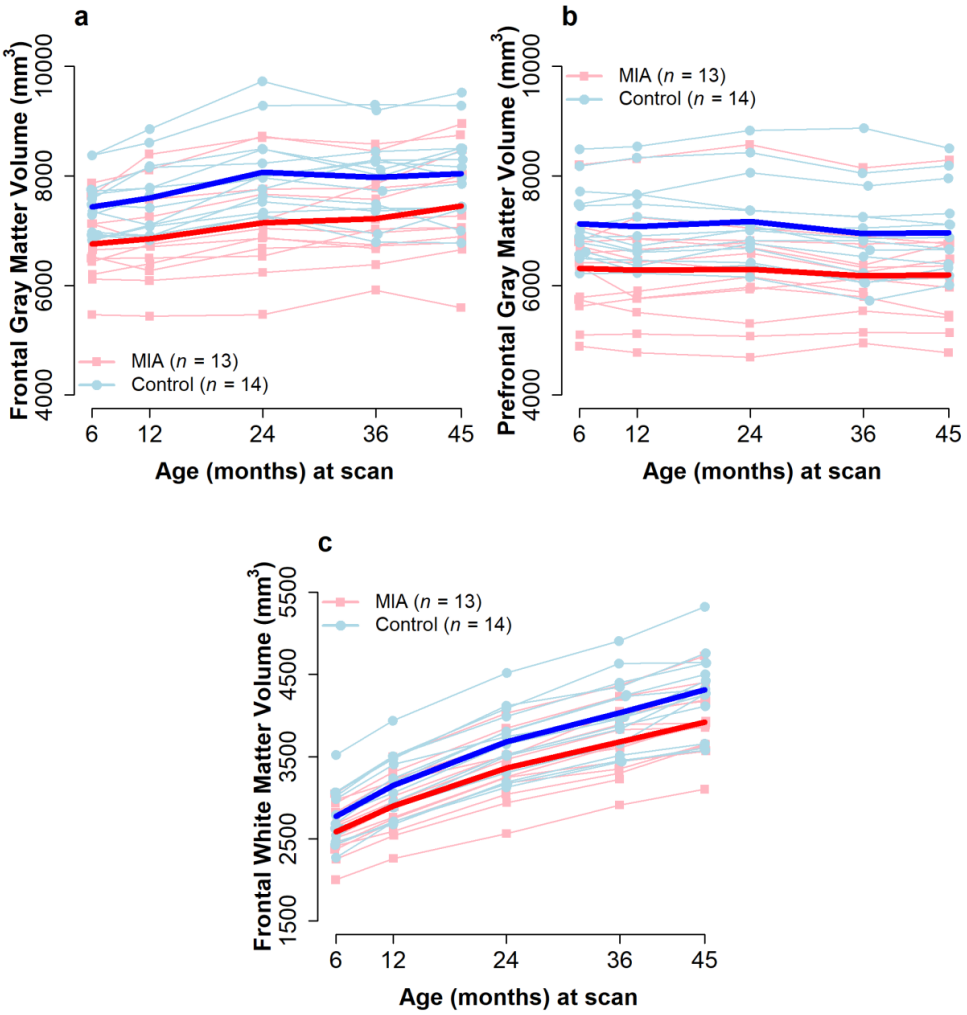


Figure 11. Brain volume trajectories for MIA-exposed and Control offspring for (a) gray matter Frontal and (b) Prefrontal regions, and (c) white matter Frontal region. The light lines represent individual trajectories and dark lines represent average values for the two groups. Gray matter group differences were significant in Frontal and Prefrontal regions, with lower volumes in MIA across all time points. For white matter, group by time interaction was significant in the Frontal region, with significantly smaller volume increases from the initial 6-month measurement to the 24-, 36-, and 45-month measurements in MIA relative to Control.



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The authors report no biomedical financial interests or potential conflicts of interest.

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Maternal Immune Activation Model Reporting Guidelines Checklist

ARRIVE Reporting Guideline & Recommendation	Arrive Item	MIA Model Specific Reporting Recommendation *Please complete this chart for each point outlined below. If not applicable, write N/A*
Study design ▶ Overview of immune activation issues For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimize the effects of subjective bias when allocating animals to treatment (e.g. randomization procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	6	MIA Specific Reporting: a. General need for improved reporting in MIA model methods + reporting pilot data ○ Details on pilot data: The MIA-induction protocols are based on our previous dosing (Weir et al., 2015) and gestational time line comparisons (Bauman et al., 2014). For the present study, the effectiveness of Poly ICLC to induce an immune response was piloted in a single non-pregnant female rhesus macaque. Immune response was measured by change in cytokine levels, specifically IL-6, in blood samples taken prior to Poly ICLC injection and 6 hours after injection. Temperature was also monitored pre-and post-injection.
Experimental procedures ▶ Compounds ▶ Validation measures For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	7	Provide details of: a. Compounds – source, vehicle, preparation/storage, administration route, volume administered, whether anesthetics were used at time of immune challenge. ○ Name of compound: Poly ICLC ○ Catalogue number: N/A ○ Lot number: N/A ○ Vehicle control used: Sterile saline ○ Route of administration: IV ○ Volume administered: ~1 ml (dose: 0.25 mg/kg, concentration: 2 mg/mL) ○ Storage conditions: Refrigerated ○ Anesthetic (type, dose, duration) used: N/A b. Housing variables at injection - temperature of room at injection time, cage change at time of injection or not ○ Light cycle of animal housing room: Lights on 0600-1800 ○ Time of day of injection: 7:30AM ○ Room temperature at injection time: 18-29 C ○ Did a cage change occur at time of injection: NO

		c. Validation of immune activation – behavior, physiological indices and/or cytokine data, including pilot dosing data ○ Method used to verify immune activation: Cytokine levels were evaluated from pre-dosing baseline blood samples, 6 hours after injection #2, 6 hours after injection #3, and post-dosing baseline blood samples. Temperature and sickness behaviors were monitored pre-dosing, throughout the dosing period, and post-dosing. d. Validation of gestational timing – vaginal plug, estrous cycle, weight gain ○ Method of validating gestational timing: Dams were from the time-mate colony at the CNPRC. Breeding was based on menses cycle, and pregnancies were confirmed and dated via ultrasound. Additional comments:
Experimental animals ▶ Species/strain/vendor a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	8	Provide details of: a. Species – considerations for appropriate species (mouse, rat, non human primate, other) ○ Species: Macaca mulatta b. Strain – variability in strain can influence model ○ Strain: N/A c. Maternal/Offspring Physiological Variables at time of immune challenge – age, body weight ○ Maternal Age at challenge: 5-13 years ○ Maternal Body weight: 5-10 kg ○ Offspring Age at challenge: N/A ○ Offspring Sex: Males only tested ○ Offspring Body weight: d. Vendor – even within the same strain, vendor can influence endpoints ○ Vendor: CNPRC breeding colony ○ Location of Vendor: ○ Room/area where animals originated from:

		Additional Comments:
Housing and husbandry ▶ Cage, ventilation, bedding, enrichment Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding program, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.	9	Provide details of: a. Caging systems ○ *At breeding* Material of cage: Stainless steel Cage dimensions: 60 x 65 x 79 cm ○ *After parturition* Material of cage: Stainless steel Cage dimensions: 60 x 65 x 79 cm ○ *At weaning* Material of cage: Stainless steel Cage dimensions: 60 x 65 x 79 cm b. Animal Holding room ○ Temperature in room: 18-29 C ○ Humidity in room: 30-70% ○ Ventilation system: 10-15 air exchanges/hour ○ Specific pathogen free [SPF]: NO ○ Are males & females housed in the same or separate rooms: Housed in same room after weaning c. Bedding exchanges/bedding type ○ Type of cage bedding used: N/A ○ Frequency of cage changes per week *during gestation:* Daily sanitation; cage change every two weeks *during neonatal period:* Daily sanitation; cage change every two w *following weaning:* Daily sanitation; cage change every two weeks d. Breeding - bred on site or timed pregnant, how many different sires (are the same fathers breeding with both experimental and control dams) Breeding location: CNPRC

		○ Gestational age at shipping: N/A ○ Biological age of dams (if not listed in Section 8c): 5-13 ○ Number of Dams bred: see below ○ How many times have dams been mated previously: 0-7 ○ How many times did the dams mate and not become pregnant: ○ Are the dams primiparous or multiparous? Dams are a mix of primi- & mult ○ What was the frequency of maternal handling during the gestational/neonatal period (e.g. cage cleanings, weighing, blood collection manipulations): i ultrasound and blood draw/trimester ○ Biological age of sires: see below ○ Number of sires bred: see below ○ How many times have sires been mated previously: see below ○ How many times did the sires mate successfully (e.g. mating resulted in pregnancy, full term birth): see below ○ If bred previously, what was the interval between mating times: ○ Are sires matched to experimental and control dams: NO ○ Describe the mating design (1:1, 1:2 etc): 1:1 e. Social enrichment – number of cage companions ○ Number of cage companions prior to breeding: 0-1 ○ Gestational age when dam separated for parturition: N/A ○ Number of cage companions at weaning: 1 f. Physical enrichment – describe enrichment devices, and when enrichment is in the cage (removed when pups born? Or present throughout study), does the enrichment type change? How frequently? ○ Describe what type of enrichment devices (and how many) are included in cage/housing room: Standard CNPRC enrichment was provided throughout the study. This included mirrors, Kong toys and other physical manipulanda, foraging boards, rotational hanging and foraging toys, coconuts, videos, and rotations in larger enclosures.

		○ Does enrichment type/access change across study? NO ○ If so, when does enrichment type/access change (e.g. enrichment removed prior to parturition and replaced in late neonatal period): Additional Comments: Social enrichment: Infants were raised in individual cages with their mothers where they had visual access to other mother-infant pairs at all times. For 3 hours each day, one familiar adult male and four familiar mother-infant pairs were allowed to freely interact in a large cage (3m l x 1.8m w x 2m h) to provide enrichment and facilitate species-typical social development. The infants were weaned from their mothers at 6 months of age and were permanently paired with a familiar peer from their rearing group. Weanlings continued the same socialization routine through approximately 18 months of age. They were transferred to the large enclosures for 3 hours each day with the same three weanlings from their rearing group, the familiar adult male, and an adult female.
Sample size ▶ Litter versus offspring a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	10	Provide details of: a. Maternal N vs offspring N ○ What is the total number of dams/litters included in the study: N=28 ○ What is the total number of offspring per litter included the study: N=28 offpsring b. Litter size and sex distribution ○ What size was each litter maintained at: ○ What age did culling take place at: ○ How many males and females were maintained in each litter: c. Cross fostering ○ Did cross fostering occur: NO ○ If so, at what age did cross fostering occur: Additional Comments:

Allocating animals to experimental groups a. Give full details of how animals were allocated to experimental groups, including randomization or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.	11	a. How many offspring per litter were used in each measure: b. Randomization/Matching procedures o What procedures were used to assign animals to groups: Dam assignment to experimental groups was balanced by age, weight, and prior conception number. Groups were filled in an alternating pattern as much as possible while taking into account the above factors. c. Sex as a biological variable (behavioral and physiological outcomes) o Were both males and females evaluated in each behavioral and physiological outcome: NO Additional Comments:
Experimental outcomes ▶ Behavioral testing ▶ Physiological endpoints Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioral changes).	12	a. Maternal behavior and pup interactions o If maternal care was evaluated, were there differences following immunogen challenge (if so, please briefly describe): N/A b. Age(s) of offspring at behavioral testing/physiological evaluation endpoints: 0-4 years of age c. Order of testing (e.g. behavioral test order) o Were animals evaluated in a counter-balanced order in terms of: *presentation of tests to each animal:* NO *order of experimental/control groups run through each test:* YES o What was the inter-test interval if a single animal underwent a battery of tests: N/A

		Additional Comments: Offspring participated in a comprehensive evaluation of brain and behavioral development from birth - four years. Behavioral data included neonatal assessments (1wk), visual paired comparison (1mos), 24 hour biobehavioral assessment (3mos), home cage and social cage observations (0-18mos), noninvasive social eye-tracking, novel cage and novel social partner observations (11, 23, 35mos), WGTA reversal learning (18mos), CANTAB (32-47mos). Magnetic resonance imaging was performed at approximately 6, 12, 24, 36, and 45 months of age and positron emission tomography (PET) imaging at 12, 24, 36, and 45 months of age. Biological samples (blood, cerebrospinal fluid, hair, feces) were collected periodically.
Statistical methods a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	13	a. Unit of analysis for each data set o Is the unit (n) of each analysis based on number of litters, or number of animals used per group: N=14 MIA-treated offspring were compared to N=14 control offspring (litter considerations do not apply). Statistical analyses were conducted within a generalized linear mixed-effects models framework that can accommodate traditional general linear models (e.g., ANOVA and multiple linear regression) for data that were assumed normally distributed and independent across individuals, as well as linear mixed-effects models for normally distributed, binary, or count data that were collected repeatedly for an individual (across time or conditions)
Other Disclosures		Please make note of any other extraneous variables that you would like to report (e.g. fire alarms, construction, temporary relocations, other variables that you think we should be considering in our studies etc.): We have adapted the MIA reporting guidelines generated for rodent MIA models for use in nonhuman primates. Additional methodological details are available upon request.

The recommended use of this reporting form is to fill it out and include it as supplemental material for each of your laboratory's research publications. If there are difficulties utilizing/adapting this fillable form, please contact one of the corresponding authors to request a copy. The authors give permission for this table to be edited for use in reporting on other animal models (e.g. postnatal immune challenge models, early life stress models) as appropriate.

Kentner AC, Bilbo AD, Brown AS, Hsiao EY, McAllister AK, Meyer U, Pearce BD, Pletnikov MV, Yolken RH, Bauman MD. (2018). Maternal immune activation: reporting guidelines to improve the rigor, reproducibility, and transparency of the model. Neuropsychopharmacology, https://doi.org/10.1038/s41386-018-0185-7.