Maternal immune activation during pregnancy alters early neurobehavioral development in nonhuman primate offspring

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ABSTRACT

Background

Human epidemiologic studies have implicated exposure to infectious or inflammatory insults during gestation in the etiology of neurodevelopmental disorders. Rodent models of maternal immune activation (MIA) have identified the maternal immune response as the critical link between maternal infection and aberrant brain and behavior development in offspring. The nonhuman primate MIA model provides an opportunity to maximize the translational utility of this model in a species more closely related to humans.

Methods

Here we evaluate the effects of MIA on brain and behavioral development in the rhesus monkey (*Macaca mulatta*). A modified form of the viral mimic, Polyinosinic-polycytidylic acid (PolyIC), was delivered to pregnant rhesus monkeys ($n=14$) in the late first trimester to stimulate a maternal immune response. Control dams received saline injections at the same gestational time points ($n=10$) or were untreated ($n=4$).

Results

MIA-treated dams exhibited a strong immune response as indexed by transient increases in sickness behavior, temperature and inflammatory cytokines. MIA-exposed offspring developed species typical milestones and demonstrate subtle changes in early in social development. However, magnetic resonance imaging demonstrated significant gray matter volume reductions in prefrontal and frontal cortices at 6, 12 and 24 months of age.

Conclusions

These findings provide new insights into the emergence of neuropathology in MIA-exposed primates and have implications for the pathophysiology of human psychiatric disorders associated with maternal gestational infection.

INTRODUCTION

The need to understand the interaction between maternal exposure to infection during pregnancy and the subsequent increased risk of central nervous system (CNS) disorders in offspring has never been more urgent[1]. Children born to women exposed to infection have an increased risk of brain disorders with neurodevelopmental origins, including both schizophrenia (SZ) and autism spectrum disorder (ASD)[2,3]. The association between maternal infection and offspring neurodevelopmental disorders documented in epidemiological studies[4-7] is further supported by the presence of infectious or inflammatory biomarkers in gestational biospecimens obtained from mothers of children later diagnosed with SZ or ASD[8-17]. The diversity of maternal infections associated with adverse offspring outcomes suggests that the maternal immune response may be the critical link between exposure to infection and altered fetal brain development. The maternal immune activation (MIA) hypothesis proposes that exposure to infection during pregnancy triggers an immune response that alters the maternal-fetal immune environment, disrupts fetal brain development, and serves as a "disease primer" for a range of neurodevelopmental and neuropsychiatric disorders in the offspring[18,19].

Preclinical models provide a translational tool to systematically explore the etiological role of prenatal immune challenge in neurodevelopmental disorders[20]. MIA models use viral mimics, such as Polyinosinic:polycytidylic acid (Poly IC), to elicit an immune response during gestation and then characterize changes in the brain and behavioral development of the offspring in a controlled environment[21]. Rodents born to MIA-treated dams exhibit a number of alterations in brain and behavioral development that resemble features of human neurodevelopmental and neuropsychiatric disease[22]. For example, impairments in offspring sensory processing and social development are highly reproducible in the rodent MIA models in spite of methodological variability[23]. Moreover, 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 neurodevelopmental disorders[24-26].

Our research team developed the first Poly IC-based nonhuman primate model to bridge the gap between rodent MIA models and human patient populations. In a previous

study, pregnant rhesus monkeys (*Macaca mulatta*) injected with a modified form of Poly IC over four days at the end of either the first or second trimester exhibited a transient but potent immune response and produced offspring that developed abnormal repetitive behaviors, reduced affiliative vocalizations and altered immune regulation within the first two years[27,28]. As they matured, monkeys exposed to MIA in the first trimester also exhibited inappropriate interactions with novel conspecifics and failed to attend to salient social cues[29]. The presence of abnormal repetitive behaviors paired with alterations in social development exhibited by MIA-treated offspring suggests that circuity relevant to neurodevelopmental disorders, including both ASD and SZ, may be vulnerable to prenatal immune challenge. Although we are at the earliest stages of exploring the underlying neuropathology in the nonhuman primate MIA model, our preliminary neuroimaging studies using positron emission tomography revealed increased striatal dopamine in late adolescence[30] and histopathological analysis revealed aberrant dendritic morphology in the dorsolateral prefrontal cortex (DLPFC)[31]. In the present study, we report developmental behavioral and neuroanatomical findings from a new and larger cohort of MIA-treated male offspring using longitudinal, multimodal neuroimaging paired with comprehensive behavioral phenotyping. We present the initial characterization of growth and development in this new cohort along with structural magnetic resonance imaging (MRI) data obtained at time roughly spanning early to mid-childhood in the rhesus monkey.

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 the psychological well-being of the animals that participated in this research. Detailed methods are provided in supplemental material.

Animal selection

Pregnant dams were selected from the indoor, time-mated breeding colony based on age, weight, parity, and number of prior live births (Supplemental Table 1). Candidate dams between 5 and 12 years old carrying a male fetus were assigned to MIA (n=14) and control/saline (n =10) (Supplemental Table 2). 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.

Maternal immune activation and validation

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 0730 hours in the cephalic vein in awake animals on gestational day (GD) 43, 44 and 46 (Supplemental Table 3). Health and behavioral observations were conducted three times pre-treatment, 6 hours after each of the three injections, and three times post-treatment. Blood was collected from the dams on approximately GD 40 while sedated for ultrasound, from awake animals on GD 44 and 46, six hours after Poly ICLC infusion, and on GD 51 or 52 while sedated for a recheck ultrasound for cytokine analysis. Blood samples were centrifuged and the serum was removed, aliquoted into 200uL 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 collected at baseline (pre-exposure at GD 40) and after the second (GD 44) and third (GD 46) Poly ICLC injections using a nonhuman primate multiplexing bead immunoassay (Milipore-Sigma, Burlington, MA) that was analyzed using the flow-based Luminex™ 100 suspension array system (Bio-Plex 200; Bio-Rad Laboratories, Inc.).

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 facilitate species-typical social development. The infants were weaned from their mothers at 6 months of age and were permanently paired with a peer from their rearing group and in the same experimental group. Weanlings continued the same socialization routine through approximately 18 months of age.

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 and 24 months of age).   The nonhuman primate offspring described in this paper are undergoing comprehensive assessments of social and cognitive development that will be the focus of future publications. Here we present data on a neonatal neurobehavioral assessment (1 week) and home cage observations of the mother-infant dyad (0-6 months), home cage observations with their age/sex/treatment matched cage mate (6-18 months) and introduction to a novel conspecific (11 months) (Supplemental Table 4). 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 our previous publications[27,32-34] and in the supplemental material.

Neuroimaging

Magnetic resonance imaging was performed under anesthesia at approximately 6, 12 and 24 months of age using a Siemens Magnetom Skyra 3-T (Davis, California) with 8-channel coil optimized for monkey brain scanning (RapidMR, Columbus, Ohio). T1 weighted images (480 sagittal slices) were acquired with TR=2500 ms, TE=3.65 ms, flip angle=7°, field of view 256x256, voxel size during acquisition 0.6x0.6x0.6 mm. Acquired images were interpolated during image reconstruction to 512x512 voxels with a final resolution of 0.3 x 0.3 x 0.3 mm. All images were analyzed by investigators who were blinded to group assignment. T1 weighted images were aligned into common atlas space[35], bias field corrected, and brain masked using AutoSeg_3.3.2[36]. 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 [37](Supplemental Figure S1). Probabilistic tissue maps from structural multi-atlas templates were applied to each subject's T1 weighted images via deformable registration. University of North Carolina (UNC) lobar parcellations were employed to parcellate the tissue segmentations into 24 lobar brain regions using the multi-atlas fusion in AutoSeg_3.3.2 (Supplemental Figure S1)[36]. For these regions, total GM and WM volumes were extracted. Lateral ventricle volumes were determined via semi-automated segmentation using the region competition deformable surface approach in ITK snap [38] as applied to the probability CSF maps from the tissue segmentation [39]. In order to limit the number of comparisons, we selected five regions of interest based on a review of literature documenting brain alterations associated with MIA: prefrontal, frontal, cingulate, temporal limbic (including amygdala and hippocampus) regions (Supplemental Figure S2), and the lateral ventricles[40-44].

Statistical analysis overview

Statistical analysis was conducted using a general linear mixed models framework that can accommodate traditional general linear models (e.g., ANOVA and multiple linear regression) for data that were assumed independent across individuals, as well as mixed-effects

linear models[45] for data that was collected repeatedly for an individual (across time or conditions). An advantage of this approach is the ability to use all available data for an individual, to account for the effect of covariates of interest, and to directly model heterogeneous variances (across groups or conditions). Transformations were employed if assumptions of the linear models were not met and nonparametric techniques (exact Wilcoxon two-sample test) were used to compare groups when transformations were unsuccessful. All models were validated both graphically and analytically. Tests were two-sided, with α = 0.05. All analyses were conducted in SAS version 9.4. (SAS Institute Inc., Cary, NC). Detailed description of the specific statistical models used to analyze each of the offspring development, physical growth, behavioral, and neuroimaging data is available in Supplemental material.

RESULTS

Validation of maternal immune activation

Blood samples collected six 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 1; Figure S4). Dams that received Poly ICLC injections also exhibited sickness behaviors, including reduced appetite and fever (Supplemental Tables 5-6 and Figure S3).

Offspring development

There were no significant differences between MIA-treated and control offspring in growth trajectories (Figure 1a, b, c, d, Supplemental Table 7), neuromotor-reflexes (Supplemental Table 8) or home cage check list observations of mother-infant and familiar infant-infant interactions (Supplemental Tables 9, 10). Subtle group differences were detected when we evaluated offspring response to a novel age/sex matched conspecific at 11 months of age (Figure 2). Table 2 summarizes the frequency and duration of the behaviors for the two groups and the results of the general linear models used to analyze them after accounting for age at testing (centered at median, 10.2 months) and stimulus animal. For most of the behaviors, there was evidence of a group by age at testing interaction. MIA infants exhibited similar durations in nonsocial and social chamber regardless of age, while older control animals tended to have longer duration in nonsocial chamber and shorter durations in the social chamber than younger animals. This pattern resulted in subtle shorter duration in nonsocial chamber and longer duration in social chamber in the MIA-treated animals than in same age controls at 10.2 months (p = .07 and .02, respectively). The same pattern of subtle group differences at older ages was present for duration of contact and proximity with stimulus animal and frequency of proximity (p = .07, .03, and .08, respectively for group differences at 10.2 months). MIA animals had consistently more entries in the social chamber (p = .09), regardless of age.

Neuroimaging

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Table 3 summarizes the volumetric measures for the two groups and Supplemental Table 11 displays the results of the mixed-effects linear models for global measures. The two groups had parallel growth trajectories from 6 to 24 months on all global measures i.e. total brain volume, total gray matter volume and total white matter volume; none of the group by time interactions reached statistical significance. Total brain volume from 6 to 24 months for the MIA-treated animals was consistently smaller than for the control animals although the difference was not significant (estimated difference [est.] = -5504 mm^3, p = .12). The same pattern of lower levels in the MIA-treated animals than in the controls was present in total gray matter (est. = -3862 mm^3, p = .11).

Supplemental Table 12 summarizes the results of the unadjusted analyses for gray and white matter volumes in the five regions of interest. For gray matter, the two groups had parallel developmental trajectories from 6 to 24 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 3), MIA-treated monkeys had lower values than controls at 6 months and these differences persisted at later ages (Frontal: est. = -382.6 mm^3, p = .009, Prefrontal: est. = -414.5 mm^3, p = .02; Supplemental Table 12). Table 4 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. = -155.3 mm^3, p = .02, Prefrontal: est. = -184.7 mm^3, p = .02).

For both frontal and prefrontal white matter, there was an interaction between time and group in unadjusted analyses. The two groups had similar levels of white matter at 6 months, but the control group tended to have larger volume increases over time, resulting in higher volumes at 24 months than the MIA group (Frontal: est. = -163.5 mm^3, p = .03, Prefrontal: est. = -128.1 mm^3, p = .04). These differences did not persist after adjusting for total brain volume.

DISCUSSION

The MIA model has emerged as a translational tool to explore the developmental pathophysiological mechanisms that link exposure to maternal infection during pregnancy with an increased risk of neurodevelopmental disorders in the offspring[20]. Although the vast majority of MIA model research has been carried out in rodents, the nonhuman primate provides a unique opportunity to evaluate the impact of MIA on brain and behavioral development in a species more closely related to humans. Our previous nonhuman primate MIA model work demonstrated that rhesus monkeys exposed to prenatal immune challenge exhibit alterations in brain and behavioral development relevant to human neurodevelopmental disorders[27-29]. Here we present initial results from a new cohort of MIA-treated nonhuman primate offspring undergoing longitudinal neuroimaging as part of a comprehensive assessment of brain and behavioral development. Structural MRI data, obtained at 6, 12 and 24 months of age, revealed deviations from species-typical brain development trajectories in the MIA-treated offspring. Compared with controls, the MIA-treated offspring exhibited reductions in frontal grey matter volumes at each time point, which roughly spans the period of early to middle human childhood. During this time, the MIA-treated offspring did not differ from controls on early assessments of physical growth and development, neonatal reflexes or preliminary evaluations of interactions with the mothers or familiar rearing partners. However, the MIA-treated offspring did exhibit subtle alterations in species-typical social behavior when introduced to a novel conspecific at approximately 11 months of age. These results provide evidence of early volumetric reductions paired with subtle alterations in social development following prenatal immune challenge in the rhesus monkey and extend the results from rodent MIA models to a primate species.

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[23]. Here we utilize a Poly IC based nonhuman primate MIA model to stimulate an inflammatory cytokine response late in the first trimester that mimics a moderate to severe infection during pregnancy. MIA-treated dams exhibited a transient immune response characterized by 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[46]. To our knowledge, this is the only nonhuman primate MIA model using Poly ICLC to stimulate the maternal immune response early in gestation, though other nonhuman primate prenatal immune challenge models have reported alterations in offspring brain and behavioral development following exposure to influenza[43] or bacterial endotoxins in the third trimester[44]. Interestingly, similar to the MIA-treated offspring in the present study, the influenza exposed offspring exhibited early reductions in grey matter volume in the absence of overt behavioral changes[43].

Volumetric reductions have also been noted in rodent MIA models[47-51]. Weiner and colleagues first documented longitudinal region-, age- and sex-specific changes in brain development in MIA-treated rat offspring using manual segmentation of regions of interest to quantify volumetric changes from postnatal day (PND) 35-90[52]. MIA-treated offspring had smaller absolute volumes of the hippocampus, striatum and prefrontal cortex and larger lateral ventricles compared to controls, though sex-specific trajectories were observed. Vernon and colleagues recently reported age-specific volumetric reductions in the anterior cingulate cortex and hippocampus of MIA-offspring using a semi-automated technique and volumetric reductions in additional cortical areas, including prefrontal cortex, using a fully automated technique to explore longitudinal changes in MIA-treated rat offspring[40]. Collectively, the rodent neuroimaging studies suggest that MIA is associated with volumetric reductions, though these changes are age, region, and sex-specific[22]. 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[22,40] and late first trimester MIA-treated nonhuman primates.

Rodent MIA models have identified numerous changes in neuronal migration, number and density, as well as alterations in dendritic structure and synapse formation that could contribute to aberrant brain growth trajectories (for review[53]). Although we are at the earliest stages of exploring neuropathology in the nonhuman primate MIA model, the gestational timing of the prenatal immune challenge may provide some insight into which stages of fetal brain development may be affected. 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 macaque gestation. Peak periods of neurogenesis for subcortical structures, including the amygdala[54] and thalamus[55], as well as the early stages of neurogenesis for the striatum[56] and hippocampus[57] occur during this time. In macaques, the early stages of corticogenesis also begin at the end of the first trimester and continue through the second trimester (GD 56-110)[58]. Exposure to prenatal immune challenge on GD 43-46 is positioned to disrupt the finely orchestrated events of neurodevelopment taking place near the end of the first trimester and could initiate a cascade of aberrant brain and behavioral development. Our preliminary evaluation of brain tissue from our earlier cohort of MIA-treated monkeys revealed subtle alterations in dendritic morphology in the DLPFC during adolescence[31]. It is not known if these subtle morphological changes in the DLPFC are present early in development or emerge as the MIA-treated offspring mature. However, the reduction in frontal cortex volume reported in the present MRI study suggests that the frontal cortex of MIA-treated offspring deviates from typical brain development trajectories in the early postnatal period and may be a particularly vulnerable region.

An emerging view is that activation of the maternal immune system during pregnancy serves as a "disease primer" that, in combination with other genetic and/or environmental risk factors, may increase the risk for specific neurodevelopmental disorders[18], including both ASD and SZ[24,59]. While comparisons between animal models and clinical disorders must be made with caution, the nonhuman primate model does more closely approximate humans in maternal immune response, placental function and offspring brain and behavioral development[25]. We have previously demonstrated that rhesus monkeys exposed to prenatal immune challenge develop aberrant behaviors after a period of early typical development[27-29] and exhibit increased striatal dopamine[30], which is a hallmark molecular biomarker of SZ[60]. The reductions in cortical grey matter volume observed in this new cohort of MIA-treated monkeys also align with neuroimaging studies from adolescents and young adults diagnosed with SZ[61-71], though at a much earlier time in postnatal development. While it is incredibly challenging to carry out adequately powered prospective neuroimaging studies in infants at risk for SZ[72], there is ample evidence from cohort studies of behavioral and cognitive changes in young children

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who go on to develop SZ[73-75]. Changes in early postnatal brain development have also been documented in other neurodevelopmental disorders associated with maternal infection, such as ASD. Early brain overgrowth has been consistently reported in young children with ASD[76-78] but findings are less consistent in older individuals. Longitudinal evaluation spanning early childhood through adolescence and adulthood are needed to better understand alterations in brain growth trajectories of both ASD and SZ.

Across species, offspring born to MIA-treated dams exhibit alterations in brain and behavioral development relevant to human neurodevelopmental and neuropsychiatric disease and supported the initial interpretation of the MIA model as an animal model "of" ASD or SZ. As the use of preclinical models continues to evolve towards a hypothesis-based approach[79], the MIA model has been highlighted in recently released NIH-guidelines (NOT-MH-19-053) as a model "for" examining the effects of prenatal immune challenge on offspring neurodevelopment. The MIA-treated monkeys in the current study did not differ from controls on measures of growth, neuromotor reflex development or interactions with their mothers of familiar rearing partners. However, subtle differences were detected in the MIA-treated animals when introduced to an unfamiliar animal at approximately 1 year of age using a modified version of the rodent 3-chamber social approach assay. The MIA-treated monkeys spent more time than controls in the large "social chamber" that contained an unfamiliar age/sex-matched conspecific temporarily confined to a small holding cage. While in the social chamber, the MIA monkeys spent more time than controls in immediate proximity (i.e., within arm's reach) to the unfamiliar animal and demonstrated trend level differences in the amount of time in contact with the unfamiliar animal's holding cage. For primates, the decision to approach and interact with another animal depends on a number of factors, including individual temperament differences paired with physical characteristics and behavior of the unfamiliar animal and the contact of the encounter[80-85]. For many species of nonhuman primates, approaching an unfamiliar conspecific or behaving impulsively with familiar animals can be met with negative outcomes such as physical aggression[86-92]. Interestingly, a similar pattern of behavior was observed in our previous pilot cohort of MIA-treated monkeys at 2 years of age[27]. The emergence of atypical social behavior in MIA-treated animals at

approximately 1 year of age suggests that the alterations in species-typical social behavior that are highly reproducible in the rodent MIA model[23] are also present in the nonhuman primate model. This will allow for the comparison of MIA downstream effects on evolutionarily conserved behavioral and biological outcome measures[93-95].

The results of the present study extend the results of rodent MIA models into a species more closely related to humans. Limitations of the current nonhuman primate study include a relatively modest sample size and the exclusion of female offspring. We recognize that sex differences are emerging as a critical factor in MIA model studies[96], and plan to next explore the nonhuman primate MIA model in female offspring. Although subtle group differences were detected at these early ages, a more comprehensive evaluation of social and cognitive development is underway along with continued longitudinal neuroimaging to characterize the emergence of neurobehavioral alterations in MIA-exposed primates. Our observation of reduced cortical volume in MIA-treated monkeys paired with subtle behavioral changes contributes to mounting evidence that early exposure to prenatal immune challenge triggers a cascade of brain and behavioral changes that are highly relevant to human neurodevelopmental and psychiatric disorders. 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.

Table 1. 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]

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

Table 2. Social Approach Paradigm. Summary of duration and frequency of behaviors for the MIA and Control animals and parameter estimates and standard errors of the general linear models assessing the relationship between group and duration and frequency of behavior variables

	MIA (n = 13)		Control (n = 14)		Difference between MIA and Control[1]	
	Mean (*SD*)	Median [Range]	Mean (*SD*)	Median [Range]	*Estimate (SE)*	*P*-value
Age (months)	10.5 (0.5)	10.6 [9.4 – 11.0]	10.1 (0.4)	10.6 [9.4 – 10.7]	0.35 (0.17)	.048
Duration of Behaviors (s)						
Total Time in Nonsocial Chamber[2]	166 (66)	168 [71 – 280]	328 (287)	298 [0 – 899]	-4.1 (2.1)	.07
Total Time in Social Chamber	733 (67)	732 [619 – 830]	572 (287)	601 [0 – 898]	181.7 (69.7)	.02
Contact with stimulus animal[2]	266 (92)	271 [93 – 418]	209 (171)	191 [0 – 514]	3.5 (1.9)	.07
Proximity with stimulus animal	275 (76)	266 [182 – 406]	182 (110)	206 [0 – 334]	83.6 (36.1)	.03
Away from stimulus animal [2]	192 (128)	172 [42 – 494]	181 (142)	170 [0 – 452]	2.8 (2.1)	.20
Frequency of Behaviors						
Proximity	44.2 (18.4)	41 [20 – 82]	31.5 (17.8)	36 [0 – 56]	12.6 (7.0)	.08
Entry Social Chamber	14.4 (6.8)	16 [5 – 27]	9.4 (9.3)	8 [0 – 33]	5.4 (3.1)	.09

Abbreviations: MIA = maternal immune activation, SE = standard error.

[1]*P*-values for age from Wilcoxon two-sample test and for behaviors from general linear models that included fixed effects for group, stimulus animal, age at testing (centered at the median, 10.2 months), and the interaction of group with age and heterogeneous variances for the two groups. The

estimate for group in these models can be interpreted as the estimated difference between 10.2-month old MIA and Control animals tested with the same stimulus animal.

[2]This variable was square-root transformed for analyses to improve its normality.

Table 3. Summary for the GM and WM volumetric measures (mm^3) from 6 to 24 months.

	6 Months		12 Months		24 Months	
	MIA	Control	MIA	Control	MIA	Control
	(n = 13)	(n = 14)	(n = 13)	(n = 14)	(n = 13)	(n = 14)
Age (days) at scan, *mean* (*SD*) [*Range*]						
	180 (2)	181 (5)	365 (2)	365 (1)	730 (1)	729 (1)
	[177-182]	[177-199]	[364-369]	[364-366]	[729-732]	[729-733]
Global Measures, *Mean* (*SD*)						
TBV	81429 (8113)	86200 (7724)	83522 (9692)	88781 (8550)	87821 (10381)	94268 (9060)
GM	59665 (6002)	63271 (5146)	59404 (6884)	63073 (5538)	60710 (7240)	65058 (5710)
WM	21764 (2245)	22929 (2666)	24118 (2889)	25708 (3133)	27111 (3270)	29210 (3598)
Left V	227 (51)	263 (112)	228 (64)	264 (142)	246 (74)	312 (147)
Right V	296 (179)	258 (82)	309 (230)	245 (72)	340 (313)	285 (73)
Frontal Measures, *Mean* (*SD*)						
Left GM	3397 (347)	3740 (253)	3439 (431)	3814 (335)	3563 (463)	4043 (365)
Right GM	3362 (328)	3693 (275)	3414 (385)	3780 (332)	3582 (451)	4025 (374)
Left WM	1308 (141)	1411 (167)	1453 (184)	1586 (191)	1710 (190)	1854 (219)
Right WM	1279 (166)	1364 (167)	1446 (169)	1566 (193)	1649 (203)	1828 (199)
Prefrontal Measures, *Mean* (*SD*)						
Left GM	3165 (449)	3570 (339)	3151 (484)	3547 (379)	3146 (501)	3588 (389)
Right GM	3148 (439)	3557 (321)	3126 (466)	3531 (381)	3153 (490)	3581 (400)
Left WM	779 (140)	840 (119)	918 (165)	1003 (138)	1070 (192)	1196 (167)
Right WM	788 (131)	862 (123)	913 (161)	1014 (147)	1081 (186)	1212 (174)
Cingulate Measures, *Mean* (*SD*)						
Left GM	1061 (132)	1138 (120)	1052 (131)	1152 (136)	1053 (150)	1145 (135)
Right GM	1091 (140)	1177 (123)	1084 (144)	1185 (119)	1084 (154)	1187 (131)
Left WM	169 (24)	191 (33)	186 (25)	209 (36)	212 (32)	242 (42)
Right WM	162 (26)	179 (27)	175 (31)	196 (32)	204 (35)	229 (38)
Temporal Limbic Measures, *Mean* (*SD*)						
Left GM	1174 (87)	1193 (111)	1278 (112)	1327 (124)	1429 (144)	1460 (142)
Right GM	1241 (113)	1249 (109)	1354 (133)	1411 (125)	1500 (178)	1557 (131)
Left WM	212 (21)	222 (24)	227 (30)	237 (32)	275 (32)	278 (36)
Right WM	216 (28)	220 (27)	236 (24)	245 (27)	258 (36)	285 (35)

Abbreviations: TBV, total brain volume; GM, gray matter; WM, white matter; V, Ventricle.

Table 4. 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	3709.8 (41.8)	<.001	3547.6 (50.5)	<.001	1176.5 (14.6)	<.001	1266.0 (15.1)	<.001
Difference (mm³) *MIA* vs. *Control*	**-155.3 (61.0)**	**.02**	**-184.7 (73.7)**	**.02**	-23.9 (20.9)	.26	33.5 (20.6)	.12
Difference (mm³) Time 2 vs. Time 1	-31.9 (17.2)	.07	-119.9 (15.1)	<.001	-28.5 (5.8)	<.001	98.1 (9.3)	<.001
Difference (mm³) Time 3 vs. Time 1	-37.4 (26.7)	.17	-298.1 (26.2)	<.001	-91.7 (9.0)	<.001	178.9 (11.9)	<.001
Difference (mm³) Left vs. Right	24.0 (13.4)	.09	11.9 (7.4)	<.001	-34.5 (9.2)	<.001	-75.3 (7.3)	<.001
Brain volume (cm³)	41.8 (2.9)	<.001	41.8 (3.1)	<.001	12.7 (1.0)	<.001	12.7 (1.1)	<.001
	White Matter Volume							
Model term	**Frontal**		**Prefrontal**		**Cingulate**		**Temporal Limbic**	
	Estimate (*SE*)	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***	**Estimate (*SE*)**	***P***
Intercept	1381.8 (20.3)	<.001	870.3 (14.5)	<.001	180.4 (3.4)	<.001	222.8 (4.2)	<.001
Difference (mm³) *MIA* vs. *Control*	-29.0 (28.0)	.32	-12.6 (21.0)	.56	-5.2 (4.9)	.30	4.3 (5.9)	.47
Difference (mm³) Time 2 vs. Time 1	130.8 (9.8)	<.001	99.8 (7.3)	<.001	8.6 (1.9)	<.001	12.5 (1.8)	<.001
Difference (mm³) Time 3 vs. Time 1	290.9 (14.0)	<.001	203.2 (10.3)	<.001	24.2 (2.6)	<.001	36.5 (2.7)	<.001
Difference (mm³) Left vs. Right	31.8 (7.9)	<.001	-10.7 (3.9)	.01	10.4 (2.2)	<.001	-1.6 (2.1)	.46
Brain volume (cm³)	17.8 (1.5)	<.001	16.4 (1.1)	<.001	3.1 (0.3)	<.001	2.7 (0.3)	<.001

Note: MIA = maternal immune activation, SE = standard error, Time 1 = 6 months, Time 2 = 12 months, Time 3 = 24 months. Mixed-effects linear regression models were fitted to 13 MIA and 14 control animals and included fixed effects for group, time and their interaction, side, brain volume, and a random effect for animal. Interactions were not retained in the reported models since the overall tests for time by group were not significant. Brain volume was centered at 86,200; thus, the intercept can be interpreted as the predicted Time 1 right volume (in mm³) for a *Control* animal with a brain of 86,200 mm³. The estimate for brain volume can be interpreted as the average increase in ROI volume (in mm³) for a 1 cm³ increase in brain volume. Statistically significant MIA vs. Control group differences (*p* < .05) bolded for emphasis.
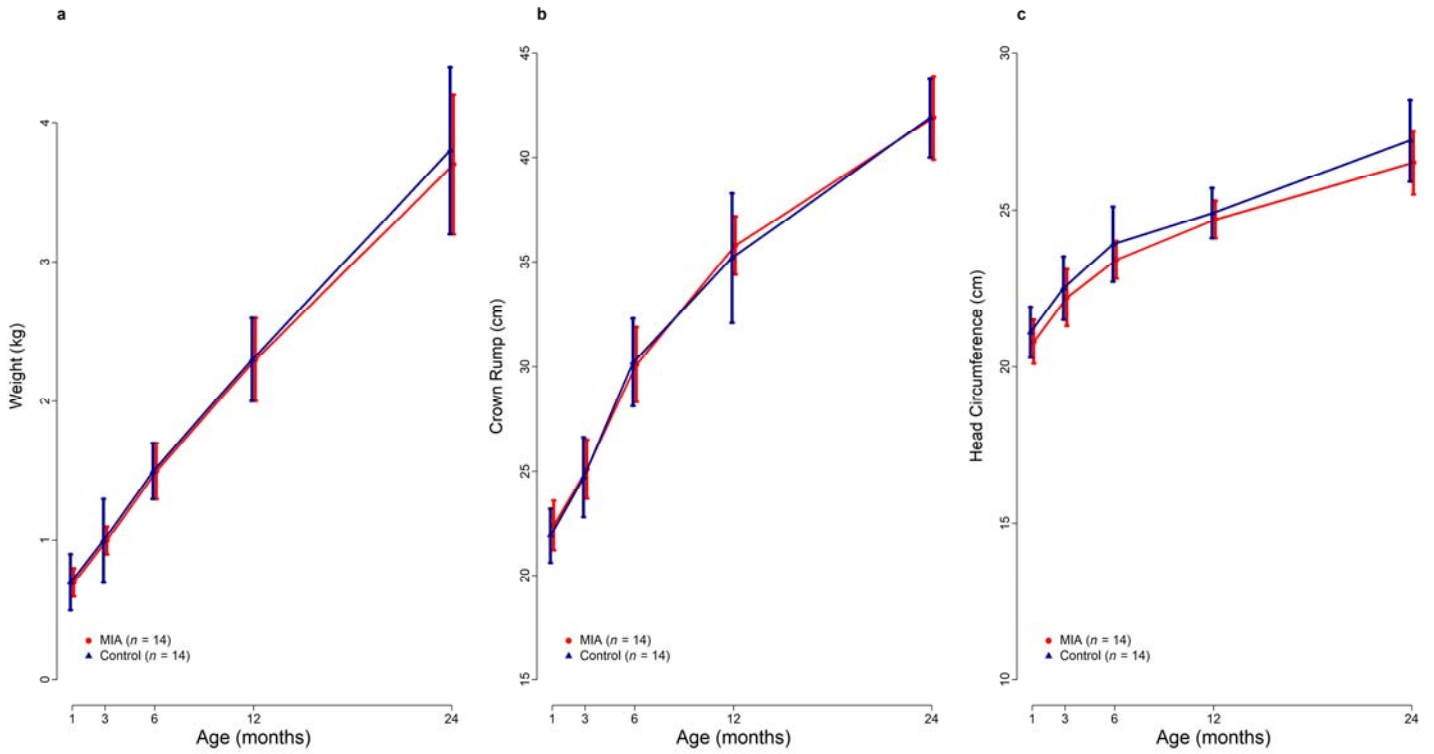
Figure 1. Average trajectory for weight (a), crown rump length (b) and head circumference (c) for the MIA and Control animals from 1 month through 24 months. Vertical bars represent 1 standard deviation.
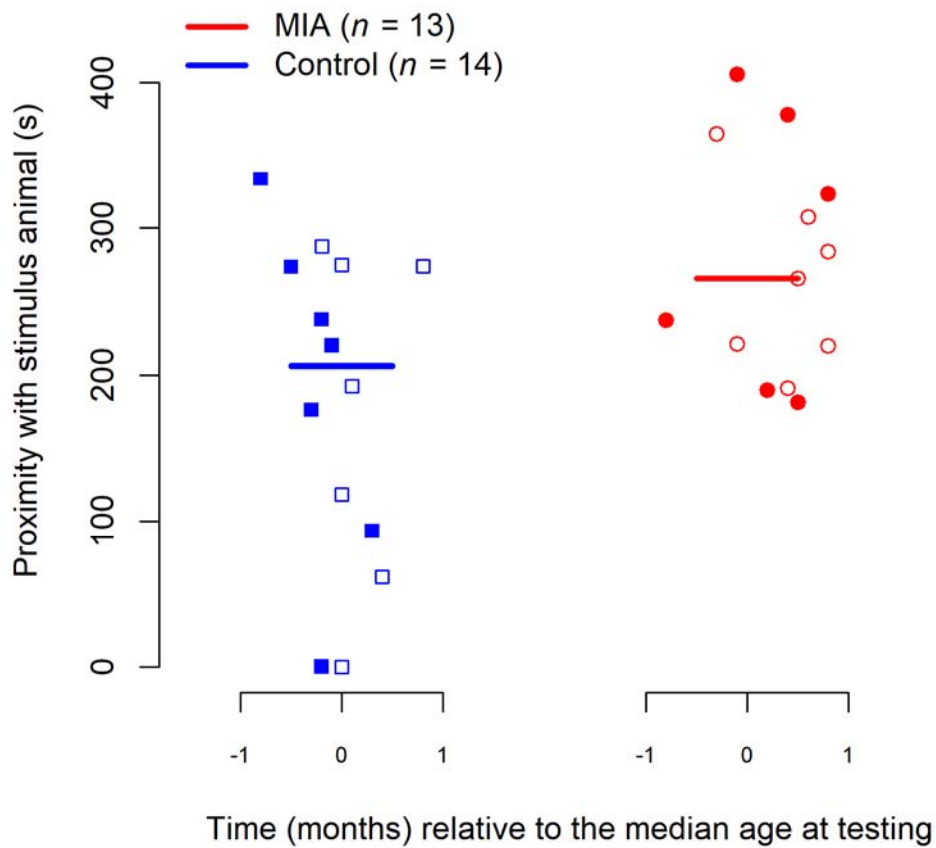
Figure 2. Duration for proximity with stimulus animal by group. Animals were tested between 9.4 and 11 months (median =10.2) using two same-sex stimulus animals (represented by filled or hollow symbols). Horizontal lines represent group medians.
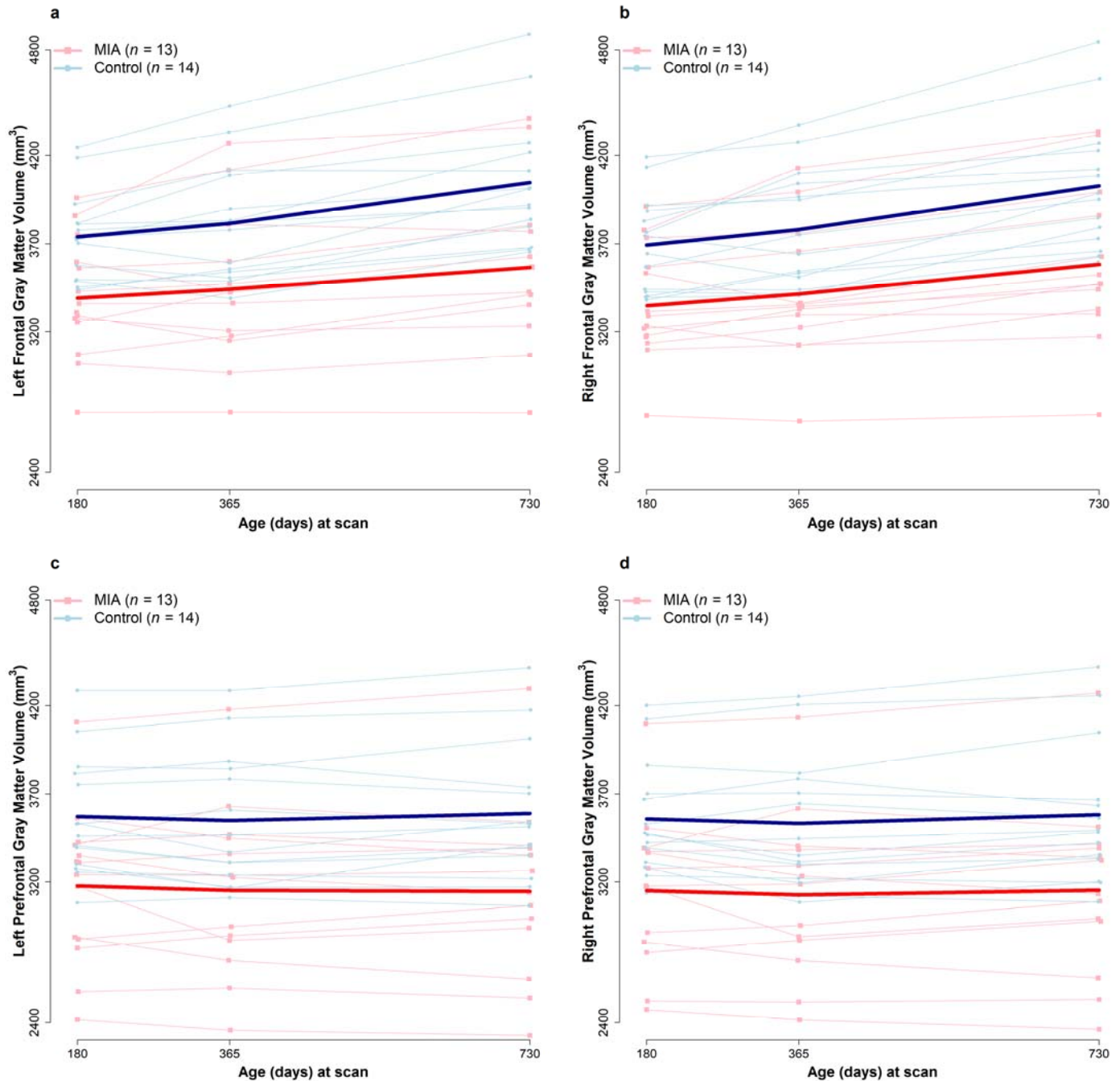
Figure 3. Gray matter volume trajectories in MIA and Control for left and right Frontal (panels a, b) and Prefrontal (panels c, d) regions. The light lines represent individual trajectories and dark lines represent average values for gray matter in the two groups.

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References

References

1   Kepinska, A. P. *et al.* Schizophrenia and Influenza at the Centenary of the 1918-1919 Spanish Influenza Pandemic: Mechanisms of Psychosis Risk. *Front Psychiatry* **11**, 72, doi:10.3389/fpsyt.2020.00072 (2020).

2   Brown, A. S. Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism. *Dev Neurobiol* **72**, 1272-1276, doi:10.1002/dneu.22024 (2012).

3   Estes, M. L. & McAllister, A. K. Maternal immune activation: Implications for neuropsychiatric disorders. *Science* **353**, 772-777, doi:10.1126/science.aag3194 (2016).

4   Blomstrom, A. *et al.* Associations Between Maternal Infection During Pregnancy, Childhood Infections, and the Risk of Subsequent Psychotic Disorder--A Swedish Cohort Study of Nearly 2 Million Individuals. *Schizophr Bull* **42**, 125-133, doi:10.1093/schbul/sbv112 (2016).

5   Atladottir, H. O. *et al.* Maternal Infection Requiring Hospitalization During Pregnancy and Autism Spectrum Disorders. *J Autism Dev Disord* **40**, 1423-1430, doi:10.1007/s10803-010-1006-y (2010).

6   Atladottir, H. O., Henriksen, T. B., Schendel, D. E. & Parner, E. T. Autism After Infection, Febrile Episodes, and Antibiotic Use During Pregnancy: An Exploratory Study. *Pediatrics* **130**, E1447-E1454, doi:10.1542/peds.2012-1107 (2012).

7   Al-Haddad, B. J. S. *et al.* Long-term Risk of Neuropsychiatric Disease After Exposure to Infection In Utero. *JAMA psychiatry*, doi:10.1001/jamapsychiatry.2019.0029 (2019).

8   Brown, A. S. *et al.* Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. *The American journal of psychiatry* **162**, 767-773, doi:10.1176/appi.ajp.162.4.767 (2005).

9   Borglum, A. D. *et al.* Genome-wide study of association and interaction with maternal cytomegalovirus infection suggests new schizophrenia loci. *Mol Psychiatry* **19**, 325-333, doi:10.1038/mp.2013.2 (2014).

10   Allswede, D. M., Buka, S. L., Yolken, R. H., Torrey, E. F. & Cannon, T. D. Elevated maternal cytokine levels at birth and risk for psychosis in adult offspring. *Schizophr Res* **172**, 41-45, doi:10.1016/j.schres.2016.02.022 (2016).

11   Canetta, S. *et al.* Elevated maternal C-reactive protein and increased risk of schizophrenia in a national birth cohort. *The American journal of psychiatry* **171**, 960-968, doi:10.1176/appi.ajp.2014.13121579 (2014).

12   Severance, E. G., Gressitt, K. L., Buka, S. L., Cannon, T. D. & Yolken, R. H. Maternal complement C1q and increased odds for psychosis in adult offspring. *Schizophr Res* **159**, 14-19, doi:10.1016/j.schres.2014.07.053 (2014).

13   Avramopoulos, D. *et al.* Infection and Inflammation in Schizophrenia and Bipolar Disorder: A Genome Wide Study for Interactions with Genetic Variation. *Plos One* **10**, doi:ARTN e0116696

10.1371/journal.pone.0116696 (2015).

14   Brown, A. S. *et al.* Elevated maternal C-reactive protein and autism in a national birth cohort. *Mol Psychiatry* **19**, 259-264, doi:10.1038/mp.2012.197 (2014).

15   Jones, K. L. *et al.* Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. *Mol Psychiatry* **22**, 273-279, doi:10.1038/mp.2016.77 (2017).

16   Abdallah, M. W. *et al.* Amniotic fluid chemokines and autism spectrum disorders: An exploratory study utilizing a Danish Historic Birth Cohort. *Brain Behavior and Immunity* **26**, 170-176, doi:10.1016/j.bbi.2011.09.003 (2012).

17   Abdallah, M. W. *et al.* Amniotic Fluid MMP-9 and Neurotrophins in Autism Spectrum Disorders: An Exploratory Study. *Autism Res* **5**, 428-433, doi:10.1002/aur.1254 (2012).

18   Meyer, U. Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems. *Biol Psychiatry* **75**, 307-315, doi:10.1016/j.biopsych.2013.07.011 (2014).

19   Knuesel, I. *et al.* Maternal immune activation and abnormal brain development across CNS disorders. *Nat Rev Neurol* **10**, 643-660, doi:10.1038/nrneurol.2014.187 (2014).

20   Brown, A. S. & Meyer, U. Maternal Immune Activation and Neuropsychiatric Illness: A Translational Research Perspective. *The American journal of psychiatry*, appiajp201817121311, doi:10.1176/appi.ajp.2018.17121311 (2018).

21   Meyer, U. & Feldon, J. To poly(I:C) or not to poly(I:C): advancing preclinical schizophrenia research through the use of prenatal immune activation models. *Neuropharmacology* **62**, 1308-1321, doi:10.1016/j.neuropharm.2011.01.009 (2012).

22   Piontkewitz, Y., Arad, M. & Weiner, I. Tracing the development of psychosis and its prevention: what can be learned from animal models. *Neuropharmacology* **62**, 1273-1289, doi:10.1016/j.neuropharm.2011.04.019 (2012).

23   Kentner, A. C. *et al.* Maternal immune activation: reporting guidelines to improve the rigor, reproducibility, and transparency of the model. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, doi:10.1038/s41386-018-0185-7 (2018).

24   Careaga, M., Murai, T. & Bauman, M. D. Maternal Immune Activation and Autism Spectrum Disorder: From Rodents to Nonhuman and Human Primates. *Biol Psychiatry* **81**, 391-401, doi:10.1016/j.biopsych.2016.10.020 (2017).

25   Phillips, K. A. *et al.* Why primate models matter. *American journal of primatology* **76**, 801-827, doi:10.1002/ajp.22281 (2014).

26   Bauman, M. D. & Schumann, C. M. Advances in nonhuman primate models of autism: Integrating neuroscience and behavior. *Experimental neurology* **299**, 252-265, doi:10.1016/j.expneurol.2017.07.021 (2018).

27   Bauman, M. D. *et al.* Activation of the maternal immune system during pregnancy alters behavioral development of rhesus monkey offspring. *Biol Psychiatry* **75**, 332-341, doi:10.1016/j.biopsych.2013.06.025 (2014).

28   Rose, D. R. *et al.* Long-term altered immune responses following fetal priming in a non-human primate model of maternal immune activation. *Brain, behavior, and immunity* **63**, 60-70, doi:10.1016/j.bbi.2016.11.020 (2017).

29   Machado, C. J., Whitaker, A. M., Smith, S. E., Patterson, P. H. & Bauman, M. D. Maternal immune activation in nonhuman primates alters social attention in juvenile offspring. *Biol Psychiatry* **77**, 823-832, doi:10.1016/j.biopsych.2014.07.035 (2015).

30   Bauman, M. D. *et al.* Preliminary evidence of increased striatal dopamine in a nonhuman primate model of maternal immune activation. *Translational psychiatry* **9**, 135, doi:10.1038/s41398-019-0449-y (2019).

31   Weir, R. K. *et al.* Preliminary evidence of neuropathology in nonhuman primates prenatally exposed to maternal immune activation. *Brain, behavior, and immunity* **48**, 139-146, doi:10.1016/j.bbi.2015.03.009 (2015).

32   Bauman, M. D. *et al.* Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey. *Translational psychiatry* **3**, e278, doi:10.1038/tp.2013.47 (2013).

33   Bauman, M. D., Lavenex, P., Mason, W. A., Capitanio, J. P. & Amaral, D. G. The development of social behavior following neonatal amygdala lesions in rhesus monkeys. *Journal of cognitive neuroscience* **16**, 1388-1411, doi:10.1162/0898929042304741 (2004).

34   Bauman, M. D., Lavenex, P., Mason, W. A., Capitanio, J. P. & Amaral, D. G. The development of mother-infant interactions after neonatal amygdala lesions in rhesus monkeys. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **24**, 711-721, doi:10.1523/JNEUROSCI.3263-03.2004 (2004).

35   Shi, Y. *et al.* UNC-Emory Infant Atlases for Macaque Brain Image Analysis: Postnatal Brain Development through 12 Months. *Frontiers in neuroscience* **10**, 617, doi:10.3389/fnins.2016.00617 (2016).

36   Wang, J. *et al.* Multi-atlas segmentation of subcortical brain structures via the AutoSeg software pipeline. *Frontiers in neuroinformatics* **8**, 7, doi:10.3389/fninf.2014.00007 (2014).

37   Cherel, M. *et al.* Automatic Tissue Segmentation of Neonate Brain MR Images with Subject-specific Atlases. *Proceedings of SPIE--the International Society for Optical Engineering* **9413**, doi:10.1117/12.2082209 (2015).

38   Yushkevich, P. A. *et al.* User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *NeuroImage* **31**, 1116-1128, doi:10.1016/j.neuroimage.2006.01.015 (2006).

39   Lyall, A. E. *et al.* Prenatal isolated mild ventriculomegaly is associated with persistent ventricle enlargement at ages 1 and 2. *Early human development* **88**, 691-698, doi:10.1016/j.earlhumdev.2012.02.003 (2012).

40   Crum, W. R. *et al.* Evolution of structural abnormalities in the rat brain following in utero exposure to maternal immune activation: A longitudinal in vivo MRI study. *Brain, behavior, and immunity* **63**, 50-59, doi:10.1016/j.bbi.2016.12.008 (2017).

41   Drazanova, E. *et al.* Poly(I:C) model of schizophrenia in rats induces sex-dependent functional brain changes detected by MRI that are not reversed by aripiprazole treatment. *Brain research bulletin* **137**, 146-155, doi:10.1016/j.brainresbull.2017.11.008 (2018).

42   Piontkewitz, Y., Arad, M. & Weiner, I. Abnormal trajectories of neurodevelopment and behavior following in utero insult in the rat. *Biol Psychiatry* **70**, 842-851, doi:10.1016/j.biopsych.2011.06.007 (2011).

43   Short, S. J. *et al.* Maternal influenza infection during pregnancy impacts postnatal brain development in the rhesus monkey. *Biol Psychiatry* **67**, 965-973, doi:10.1016/j.biopsych.2009.11.026 (2010).

44   Willette, A. A. *et al.* Brain enlargement and increased behavioral and cytokine reactivity in infant monkeys following acute prenatal endotoxemia. *Behavioural brain research* **219**, 108-115, doi:10.1016/j.bbr.2010.12.023 (2011).

45   Laird, N. M. & Ware, J. H. Random-effects models for longitudinal data. *Biometrics* **38**, 963-974 (1982).

46   Smith, S. E., Li, J., Garbett, K., Mirnics, K. & Patterson, P. H. Maternal immune activation alters fetal brain development through interleukin-6. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **27**, 10695-10702, doi:10.1523/JNEUROSCI.2178-07.2007 (2007).

47   Piontkewitz, Y., Assaf, Y. & Weiner, I. Clozapine administration in adolescence prevents postpubertal emergence of brain structural pathology in an animal model of schizophrenia. *Biol Psychiatry* **66**, 1038-1046, doi:10.1016/j.biopsych.2009.07.005 (2009).

48   Richetto, J. *et al.* Genome-Wide Transcriptional Profiling and Structural Magnetic Resonance Imaging in the Maternal Immune Activation Model of Neurodevelopmental Disorders. *Cerebral cortex* **27**, 3397-3413, doi:10.1093/cercor/bhw320 (2017).

49   Li, Q. *et al.* Voxel-based analysis of postnatal white matter microstructure in mice exposed to immune challenge in early or late pregnancy. *NeuroImage* **52**, 1-8, doi:10.1016/j.neuroimage.2010.04.015 (2010).

50   Li, Q. *et al.* Prenatal immune challenge is an environmental risk factor for brain and behavior change relevant to schizophrenia: evidence from MRI in a mouse model. *Plos One* **4**, e6354, doi:10.1371/journal.pone.0006354 (2009).

51   Fatemi, S. H. *et al.* Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: implications for genesis of neurodevelopmental disorders. *Schizophr Res* **99**, 56-70, doi:10.1016/j.schres.2007.11.018 (2008).

52   Piontkewitz, Y., Arad, M. & Weiner, I. Risperidone administered during asymptomatic period of adolescence prevents the emergence of brain structural pathology and behavioral abnormalities in an animal model of schizophrenia. *Schizophr Bull* **37**, 1257-1269, doi:10.1093/schbul/sbq040 (2011).

53   Bergdolt, L. & Dunaevsky, A. Brain changes in a maternal immune activation model of neurodevelopmental brain disorders. *Progress in neurobiology*, doi:10.1016/j.pneurobio.2018.12.002 (2018).

54   Kordower, J. H., Piecinski, P. & Rakic, P. Neurogenesis of the amygdaloid nuclear complex in the rhesus monkey. *Brain research. Developmental brain research* **68**, 9-15 (1992).

55   Ogren, M. P. & Racic, P. The prenatal development of the pulvinar in the monkey: 3H-thymidine autoradiographic and morphometric analyses. *Anat Embryol (Berl)* **162**, 1-20 (1981).

56   Brand, S. & Rakic, P. Genesis of the primate neostriatum: [3H]thymidine autoradiographic analysis of the time of neuron origin in the rhesus monkey. *Neuroscience* **4**, 767-778 (1979).

57   Rakic, P. & Nowakowski, R. S. The time of origin of neurons in the hippocampal region of the rhesus monkey. *J Comp Neurol* **196**, 99-128, doi:10.1002/cne.901960109 (1981).

58   Rakic, P. Specification of cerebral cortical areas. *Science* **241**, 170-176 (1988).

59   Meyer, U. & Feldon, J. Epidemiology-driven neurodevelopmental animal models of schizophrenia. *Progress in neurobiology* **90**, 285-326, doi:10.1016/j.pneurobio.2009.10.018 (2010).

60   Weinstein, J. J. *et al.* Pathway-Specific Dopamine Abnormalities in Schizophrenia. *Biol Psychiatry* **81**, 31-42, doi:10.1016/j.biopsych.2016.03.2104 (2017).

61   van Haren, N. E. *et al.* Changes in cortical thickness during the course of illness in schizophrenia. *Archives of general psychiatry* **68**, 871-880, doi:10.1001/archgenpsychiatry.2011.88 (2011).

62   Sun, D. *et al.* Progressive brain structural changes mapped as psychosis develops in 'at risk' individuals. *Schizophr Res* **108**, 85-92, doi:10.1016/j.schres.2008.11.026 (2009).

63   Satterthwaite, T. D. *et al.* Structural Brain Abnormalities in Youth With Psychosis Spectrum Symptoms. *JAMA psychiatry* **73**, 515-524, doi:10.1001/jamapsychiatry.2015.3463 (2016).

64   Rapoport, J. L., Giedd, J. N. & Gogtay, N. Neurodevelopmental model of schizophrenia: update 2012. *Mol Psychiatry* **17**, 1228-1238, doi:10.1038/mp.2012.23 (2012).

65   Lawrie, S. M. *et al.* Brain structure, genetic liability, and psychotic symptoms in subjects at high risk of developing schizophrenia. *Biol Psychiatry* **49**, 811-823 (2001).

66   Dazzan, P. *et al.* Volumetric abnormalities predating the onset of schizophrenia and affective psychoses: an MRI study in subjects at ultrahigh risk of psychosis. *Schizophr Bull* **38**, 1083-1091, doi:10.1093/schbul/sbr035 (2012).

67   Job, D. E., Whalley, H. C., Johnstone, E. C. & Lawrie, S. M. Grey matter changes over time in high risk subjects developing schizophrenia. *NeuroImage* **25**, 1023-1030, doi:10.1016/j.neuroimage.2005.01.006 (2005).

68   Chung, Y. *et al.* Prodromal Symptom Severity Predicts Accelerated Gray Matter Reduction and Third Ventricle Expansion Among Clinically High Risk Youth Developing Psychotic Disorders. *Molecular neuropsychiatry* **1**, 13-22, doi:10.1159/000371887 (2015).

69   Chung, Y. *et al.* Ventricular enlargement and progressive reduction of cortical gray matter are linked in prodromal youth who develop psychosis. *Schizophr Res* **189**, 169-174, doi:10.1016/j.schres.2017.02.014 (2017).

70   Cannon, T. D. *et al.* Progressive reduction in cortical thickness as psychosis develops: a multisite longitudinal neuroimaging study of youth at elevated clinical risk. *Biol Psychiatry* **77**, 147-157, doi:10.1016/j.biopsych.2014.05.023 (2015).

71   Steen, R. G., Mull, C., McClure, R., Hamer, R. M. & Lieberman, J. A. Brain volume in first-episode schizophrenia: systematic review and meta-analysis of magnetic resonance imaging studies. *Br J Psychiatry* **188**, 510-518, doi:10.1192/bjp.188.6.510 (2006).

72   Gilmore, J. H. *et al.* Prenatal and neonatal brain structure and white matter maturation in children at high risk for schizophrenia. *The American journal of psychiatry* **167**, 1083-1091, doi:10.1176/appi.ajp.2010.09101492 (2010).

73   Fusar-Poli, P. *et al.* The psychosis high-risk state: a comprehensive state-of-the-art review. *JAMA psychiatry* **70**, 107-120, doi:10.1001/jamapsychiatry.2013.269 (2013).

74   Haroun, N., Dunn, L., Haroun, A. & Cadenhead, K. S. Risk and protection in prodromal schizophrenia: ethical implications for clinical practice and future research. *Schizophr Bull* **32**, 166-178, doi:10.1093/schbul/sbj007 (2006).

75   Cannon, T. D. How Schizophrenia Develops: Cognitive and Brain Mechanisms Underlying Onset of Psychosis. *Trends in cognitive sciences* **19**, 744-756, doi:10.1016/j.tics.2015.09.009 (2015).

76   Shen, M. D. *et al.* Early brain enlargement and elevated extra-axial fluid in infants who develop autism spectrum disorder. *Brain* **136**, 2825-2835, doi:10.1093/brain/awt166 (2013).

77   Hazlett, H. C. *et al.* Early brain development in infants at high risk for autism spectrum disorder. *Nature* **542**, 348-351, doi:10.1038/nature21369 (2017).

78   Nordahl, C. W. *et al.* Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 20195-20200, doi:10.1073/pnas.1107560108 (2011).

79   Gordon, J. A. A Hypothesis-Based Approach: The Use of Animals in Mental Health Research. *NIMH Director's Message* (2019).

80   Weinstein, T. A. & Capitanio, J. P. Individual differences in infant temperament predict social relationships of yearling rhesus monkeys, Macaca mulatta. *Behaviour* **75**, 262-300 (2008).

81   McCowan, B. *et al.* Network stability is a balancing act of personality, power, and conflict dynamics in rhesus macaque societies. *Plos One* **6**, e22350, doi:10.1371/journal.pone.0022350 (2011).

82   Capitanio, J. P. Individual differences in emotionality: social temperament and health. *American journal of primatology* **73**, 507-515, doi:10.1002/ajp.20870 (2011).

83   Suomi, S. J. Early determinants of behaviour: evidence from primate studies. *British medical bulletin* **53**, 170-184 (1997).

84   Novak, M. A. & Suomi, S. J. Social interaction in nonhuman primates: an underlying theme for primate research. *Laboratory animal science* **41**, 308-314 (1991).

85   Capitanio, J. P. Personality dimensions in adult male rhesus macaques: Prediction of behaviors across time and situation. *American journal of primatology* **47**, 299-320 (1999).

86   Fairbanks, L. A. Individual differences in response to a stranger: social impulsivity as a dimension of temperament in vervet monkeys (Cercopithecus aethiops sabaeus). *J Comp Psychol* **115**, 22-28 (2001).

87   Fairbanks, L. A. *et al.* Adolescent impulsivity predicts adult dominance attainment in male vervet monkeys. *American journal of primatology* **64**, 1-17, doi:10.1002/ajp.20057 (2004).

88   Fairbanks, L. A., Melega, W. P., Jorgensen, M. J., Kaplan, J. R. & McGuire, M. T. Social impulsivity inversely associated with CSF 5-HIAA and fluoxetine exposure in vervet monkeys. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **24**, 370-378, doi:10.1016/S0893-133X(00)00211-6 (2001).

89   Manuck, S. B., Kaplan, J. R., Rymeski, B. A., Fairbanks, L. A. & Wilson, M. E. Approach to a social stranger is associated with low central nervous system serotonergic responsivity in female cynomolgus monkeys (Macaca fascicularis). *American journal of primatology* **61**, 187-194, doi:10.1002/ajp.10118 (2003).

90   Fairbanks, L. A. *et al.* Genetic contributions to social impulsivity and aggressiveness in vervet monkeys. *Biol Psychiatry* **55**, 642-647, doi:10.1016/j.biopsych.2003.12.005 (2004).

91   Westergaard, G. C. *et al.* Physiological correlates of aggression and impulsivity in free-ranging female primates. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **28**, 1045-1055, doi:10.1038/sj.npp.1300171 (2003).

92   Kinnally, E. L., Whiteman, H. J., Mason, W. A., Mendoza, S. P. & Capitanio, J. P. Dimensions of response to novelty are associated with social engagement and aggression in adult male rhesus macaques (Macaca mulatta). *J Comp Psychol* **122**, 195-203, doi:10.1037/0735-7036.122.2.195 (2008).

93   Stewart, A. M. & Kalueff, A. V. Developing better and more valid animal models of brain disorders. *Behavioural brain research* **276**, 28-31, doi:10.1016/j.bbr.2013.12.024 (2015).

94   Belzung, C. & Lemoine, M. Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biol Mood Anxiety Disord* **1**, 9, doi:10.1186/2045-5380-1-9 (2011).

95   Ryan, A. M., Berman, R. F. & Bauman, M. D. Bridging the species gap in translational research for neurodevelopmental disorders. *Neurobiol Learn Mem* **165**, 106950, doi:10.1016/j.nlm.2018.10.006 (2019).

96   Coiro, P. & Pollak, D. D. Sex and gender bias in the experimental neurosciences: the case of the maternal immune activation model. *Translational psychiatry* **9**, 90, doi:10.1038/s41398-019-0423-8 (2019).