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Article

Impact of Maternal Immune Activation Early in Pregnancy on Brain Development of Offspring: A Combined Morphological, Spectroscopic, and Behavioral Study

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Abstract: Serological human birth cohort studies have identified maternal infection during pregnancy as a risk factor for development of disorders such as Autism Spectrum Disorder and schizophrenia in offspring. Similarly, in experiments using animal models, maternal immune activation (MIA) has been shown to alter neuroanatomical and behavioral development in offspring. This study employs magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) in conjunction with behavioral assays to refine our understanding of the impact of MIA on neurobiological development in exposed animals. On gestational day nine, pregnant dams were injected with either polyinosinic:polycytidylic acid (POL) to induce MIA or saline (SAL) as a control. Whole-brain MRI, localized proton MRS, and behavioral tests (open field, three chambered social approach, and prepulse inhibition) were acquired at two timepoints, during adolescence (postnatal day [PND] 35) and adulthood (PND 60).

Whole-brain voxel-wise volumetric analyses revealed that MIA offspring exhibited altered volume in the hippocampus and caudate putamen (CPu) between adolescence and early adulthood. MRS data were assessed at each timepoint separately; MIA offspring during early adulthood but not adolescence exhibited trending reductions in γ-aminobutyrate (GABA) ($p = 0.06$) and myo-inositol (Ins) ($p = 0.08$) compared to saline controls. However, these metabolite differences did not reach levels of significance, even before multiple comparison corrections. Open field testing revealed that during adolescence, MIA offspring displayed a more anxious phenotype than controls wherein they spent less time in the anxiogenic center zone of the open field arena ($p < 0.007$), but this difference normalized by adulthood. There were no significant differences in sociability preference, novelty preference, or prepulse inhibition comparing the groups.

Results suggest that early gestational exposure to MIA results in subtle neuroanatomical changes in the trajectories of development, trending behavioral changes in adolescent offspring, and slight neurochemical changes in young adult offspring. Maternal infection alone may not be enough; additional genetic or environmental risk factors may be required to elicit the more typical symptoms of neuropsychiatric disorders.

Keywords: structural MRI; MRS; maternal immune activation; altered trajectories

1. Introduction

Evidence from human and preclinical studies suggests that exposure to maternal infection *in utero* can alter the development of offspring, acting as a risk factor for the emer-

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gence of psychiatric disorders in humans and altering brain structure and behavior in rodents (Estes and McAllister 2016; Guma et al. 2021). These effects have been documented following maternal infection with influenza, measles, mumps, chickenpox, and polio (Estes and McAllister 2016), suggesting maternal immune activation (MIA) and not the pathogenic source is likely responsible for altered neurodevelopment (Solek et al. 2018). Birth cohort studies assessing maternal blood serum for immune biomarkers relate prenatal exposure to pro-inflammatory cytokines associated with infection to increased risk for Autism Spectrum Disorder (ASD) in early childhood and schizophrenia in early adulthood (Brown 2012). With behavioral tasks, rodent experiments shed light on the specific effects of MIA independent of other risk factors, recapitulating ASD- (Malkova et al. 2012) and psychosis-relevant (Gogos et al. 2020) behaviors. Studies in non-human animals critically contribute to a deeper understanding of the cellular circuits underlying altered developmental trajectories, attempting to provide a link to human studies in individuals at risk for psychiatric disorders (Mueller et al. 2020; Duchatel et al. 2016; Hui et al. 2020).

Magnetic resonance (MR) techniques can be leveraged to assess the long-term impact of MIA, both in humans and non-human animals, longitudinally, and across anatomical, functional, and neurochemical dimensions (Guma, Plitman, and Chakravarty 2019; Chakravarty and Guma 2021). Integrating these techniques with behavioral data allows for a comprehensive characterization of the long-term effects of early life environmental exposures on neurodevelopment, such as MIA (Mueller et al. 2020; Guma et al. 2021; Qi Li et al. 2009), but prior studies using these assays have yielded inconsistent results. For example, while some report alterations in N-acetylaspartate levels in the anterior cingulate area (ACA) (Q. Li et al. 2015), or glutathione and taurine in the prefrontal cortex (Vernon et al. 2015), others have not identified significant differences (Vigli et al. 2020). In previous work we took a multivariate statistical approach to identify the impact of MIA on patterns of changes between brain volumes and behavioral metrics (Guma et al. 2021). In this study, we expand previous work by adding MRS experiments, allowing for a comprehensive analysis of neurochemistry, neuroanatomy, and behavior to understand where individual subjects fall on a continuum differing from controls, contrasting the categorical approach employed by some studies (Mueller et al. 2020).

Our previous work examined the impact of MIA exposure at gestational day (GD) 9 and 17 on neurodevelopment through the lifespan in mice. We observed alterations specific to GD 9 exposure in hippocampal, striatal, and anterior cingulate cortex development, particularly from adolescence (PND 35) to early adulthood (PND 60) (Guma et al. 2021). Based on these previous observations, the current work seeks to further our understanding of the relationship between anatomy, neurochemistry, and behavior in this vulnerable window. Specifically, we integrate MRI-derived volumetric data and neurochemical data (MRS in the ACA) at two timepoints (postnatal day [PND] 35 and 60), followed by behavioral tests assaying anxiety, sociability, and sensorimotor gating. Based on our previous study, we predicted MIA exposure to induce altered volumetric trajectory of the hippocampus, cingulate cortex, striatum, subiculum, nucleus accumbens, septal nucleus, periaqueductal gray, and cerebellar vermis/Crus I (Guma et al. 2021). Based on previous MRS experiments investigating the impact of MIA on the ACA, we predicted increased N-acetylaspartate (NAA) and decreased Ins, potentially reflecting disruptions to oligodendrocytes and astrocytes (Q. Li et al. 2015). Behaviourally, we predicted increased anxiety, decreased sociability, and impaired sensorimotor gating in MIA-exposed offspring.

2. Materials and Methods

2.1. Animals

Figure 1 represents the experimental timeline. Animals were treated in accordance with the Canadian Council on Animal Care and approved by the McGill University Animal Care Committee (Montreal, QC, Canada). Twelve female and nine male C57BL/6J

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mice were used for timed mated breeding. Ten male and ten female offspring were included per condition. Dams were housed individually under standard laboratory conditions in a temperature- (22∓ 2 ℃) and humidity- (55∓10%) controlled room on a 12 h light-dark cycle (lights on at 8:00 am) with standard food and water available *ad libitum*.

2.2. Maternal Immune Activation

Male and female mice were combined in the late afternoon, and successful copulation was confirmed the following morning by presence of a vaginal plug, marking GD 0 (maximally combined for 3 nights, given the absence of a plug). MIA was induced in pregnant mice early in pregnancy, on GD 9, by intraperitoneal (i.p.) injection of 5 mg/kg (0.1 mL/10 g) of polyinosinic-polycytidylic acid potassium salt (POL; n=6 dams; P9582 Millipore-Sigma, Canada) diluted in sterile saline, while control dams received an injection of 0.9% sterile saline (SAL; n=6 dams). We validated the impact of the POL in a separate cohort of dams (n=7) with an enzyme-linked immunosorbent assay (Supplementary Methods [SM] 1.1), confirming that same-day POL injection increased cytokine response relative to SAL (sTable 1, sFig 1). Exposure at GD 9 predisposes the fetus to future developmental abnormalities given that migration and synapse formation occur during this point in the gestational period (Meyer, Yee, and Feldon 2007). Pups were weaned at PND 21 and where possible, two males and two females (STable 2, SM 1.2) were included from each litter in the experiment, with time points indicated in Fig 1.

2.3. Magnetic Resonance Methods

All MRI images were acquired *in vivo* at 7.0T using a Bruker Biospec 70/30 scanner (Bruker, Billerica, MA, United States) and cryogenically-cooled surface coil (mouse 1H Quadrature Transmit/receive MRI CryoProbe for 10.5 cm Gradients and Larger) at the Douglas Research Centre's Brain Imaging Centre. Mice were anaesthetized (induced 5%, maintained 1-2% isoflurane in oxygen gas) to maintain a breathing rate between 70-100 breaths per minute, adjusted manually (Fresenius Kabi Canada Ltd., Richmond Hill, CA). Warm air (37 ℃) was blown into the bore of the scanner to maintain a constant body temperature (SA instruments, Inc., monitoring system, Stony Brook, NY, United States).

2.3.1. MRI Acquisition and Processing

First, three dimensional T1-weighted images were acquired with the following parameters: fast low-angle shot [FLASH] with two averages; repetition time (TR)/echo time (TE) = 21.55 ms/5.13 ms, matrix size = 260 x 158 x 210, voxel dimensions = 70 μm isotropic, flip angle = 20°, 23 min total. A functional sequence was acquired with a gradient-echo echo planar imaging sequence (EPI; in-plane resolution 0.25 x 025 mm, 0.5 mm slice thickness, TR/TE = 1000 ms/15 ms, matrix size = 96 x 40, voxel dimensions = 0.25 mm x 0.25 mm, ~8 min total). As we could not identify meaningful networks based on established rodent imaging paradigms, they were not used for this study (SM 1.3) (Grandjean et al. 2020; Belloy et al. 2018; Grandjean et al. 2014).

Structural images (n=80) were exported as Digital Imaging and Communications in Medicine (DICOM) files, converted to the Medical Imaging NetCDF (MINC) format, preprocessed to enable downstream analyses, and visually inspected for quality control (QC; https://github.com/CoBrALab/documentation/wiki/Mouse-QC-Manual-(Structural)). Following quality control, three scans were excluded from analyses (all from the second timepoint, one POL male, one POL female, and one SAL male). Preprocessing steps included first stripping images of their native coordinate system. The images were denoised with a patch-based adaptive non-local means algorithm (Coupe et al. 2008; Manjón et al. 2010). Next they were affinely registered to an average mouse template to produce a rough brain mask. To correct for bias-field intensity inhomogeneities, the images were corrected using N4ITK (Tustison et al. 2010) at a minimum spline distance of 5 mm. The final step

 doi:10.20944/preprints202203.0136.v1

included applying an affine registration (descaled and desheared) to apply a grid-preserving rigid resample transforming the corrected scan into the mouse template space. Preprocessed images were used as inputs for the in-house two-level deformation based morphometry pipeline to perform registration as in previous studies from our group (Kong et al. 2018; Guma et al. 2021; Gallino et al. 2019; Rollins et al. 2019; https://github.com/cobralab/twolevel_ants_dbm).

In brief, in the first level, affine and non-linear iterative registration was used to create a subject-average per mouse (by registering all scans for one mouse using a group-wise averaging technique) (Avants et al. 2011). In the second level, all subject averages were iteratively registered to create a study average and a common space for statistical analysis. Within-subject absolute log-transformed Jacobian determinants (Chung et al. 2001) were extracted as a whole-brain, voxel-wise measure of volume change to be used in statistical analyses (see Section 2.6). Prior to analyses, determinants were blurred using a 0.2 mm Gaussian kernel (van Eede et al. 2013). Quality control (QC) was performed through visual inspection to ensure that registrations worked as expected (*Wiki_Documentation:MouseQC* n.d.). Linear mixed effects models (LMER's) were used to model both fixed effects (interaction between age and treatment; sex) and random intercepts (subject number; litter), with the parameters selected following comparison with Akaike Information Criteria (SM 1.4).

2.3.2. MRS Acquisition and Processing

MRS was acquired from a 1.2 x 2.6 x 2.5 mm^3 voxel in the ACA with a Point Resolved Spectroscopy sequence (PRESS; TR/TE=3000/8.5 ms, 256 averages). Automated localized shimming was performed using the FASTMAP method (Gruetter 1993) (ParaVision 5.1, Bruker). Specifically, first and second order shims were first optimized on a 5 x 5 x 5 mm^3 voxel, followed by first order-only shimming on a smaller, local 3.5 x 3.5 x 3.5 mm^3 voxel, both surrounding the region of interest (Fowler et al. 2020). Eight averages were acquired without VAPOR water suppression (Tkác et al. 1999) for eddy current correction and as a reference for absolute metabolite quantification prior to acquiring 256 averages with water suppression. Before acquiring the data, shimming was manually refined over the predetermined voxel to obtain water line widths of <12 Hz (Vernon et al. 2015). Total time in the scanner varied from ~40 min to ~1.25 hours based on the manual shim process.

All spectra were preprocessed with the FID Appliance (FID-A) toolbox (Simpson et al. 2017) in MATLAB (version R2019a, The MathWorks, Inc., Natick, Massachusetts, United States). Spectra were compared before and after preprocessing (removal of motion-corrupted averages, frequency drift correcting, time-domain truncation, and autophasing), and the better of the two spectra (based on a ratio of maximum peak height divided by minimum full-width half-maximum [FWHM]) was used for quantification. Spectra were analyzed using the linear combination analysis method LCModel (version 6.3, Stephen Provencher Inc, Oakville, Ontario, Canada) using a basis set containing eighteen individual metabolite basis spectra simulated in FID-A (SM 1.5.1). Absolute quantification was performed using the unsuppressed water signal as a reference.

Quality control was performed following expert recommendations (Kreis 2016) to exclude either entire subjects or entire metabolites. On the subject level, exclusion criteria were: a) subjects where more than 20% of the metabolites were undetectable (excluded n=1), b) subjects where the average CRLB (excluding undetected samples) was greater than 20 (excluded n=2), and c) metabolites where the metabolite was undetectable for 10% of the sample and the average CRLB (excluding undetected samples) was greater than 20 (excluded metabolites: lactate (Lac), glucose (Glc), glycerophosphocholine (GPC), and NAAG.

Data from test-retest using an identical acquisition protocol were used to determine which metabolites were the most reliable between two acquisitions (SM 1.5.2). For six adult mice (three males, three females), MRS data were preprocessed and quantified identically as for the experimental data. Percent change was calculated for each metabolite

 doi:10.20944/preprints202203.0136.v1

between time 1 and time 2 for each mouse, then averaged across the 6 mice. Metabolites with an average percent change between -10% and 10% were selected as the most reliable to be included in the analysis of the experimental data. These included creatine (cr), phosphocreatine (PCr), GABA, glutamine (gln), Ins, NAA, and taurine (Tau) (sFig. 2).

2.4. Behavioral Tests

Three behavioral tests were collected following each scan with at least two days of rest between each test, administered in the following order: open field test (OFT), three-chamber social approach task, and prepulse inhibition (PPI). All tests were preceded by ~30 minute habituation to the testing room. Videos were acquired and processed offline with the Ethovision XT 12 tracking system (Noldus Information Technology, Leesburg, VA, USA).

In brief, the OFT was used to assess anxiety-like behavior (Kraeuter et al. 2019) by examining the ratio of frequency of passes and distance traveled in the center of the open field and the outer perimeter (edges/corners) (SM 1.6.1). The three chambered social approach task was used to assess sociability (Yang et al. 2011) comparing duration of time and frequency of passes through 1) a "social" versus "object" chamber and 2) a new versus familiar intruder (SM 1.6.2). Finally, sensorimotor gating was assessed with PPI of startle response to acoustic stimuli (SM 1.6.3.) (Swerdlow et al. 1994).

At PND 35, data were analyzed from nineteen POL offspring (9 females, 10 males), and twenty SAL offspring (10 females, 10 males). Unfortunately, due to the circumstances of the COVID-19 pandemic during 2020, not all behavioral data at PND 60 could be acquired (fifteen scans completed with exemption for research shut-down) and the second timepoint behavioral analyses include data from only fifteen POL offspring (6 females, 9 males) and twenty SAL offspring (10 females, 10 males).

2.6. Statistical Analyses

Longitudinal analyses.

The study was designed to be powered for longitudinal volumetric analyses (details in SM 1.7). Therefore, all whole-brain, voxelwise anatomical analyses were modelled longitdudinally with LMERs, and results are reported from the interaction between treatment (POL or SAL) and age in days, including sex as a covariate (full model in SM 1.4). Subject identification (ID) and litter were included as random intercepts to account for the repeated subject measures and the nested litters. Structural analyses were performed on the log-transformed within-subject absolute Jacobian determinant to capture overall change in brain volume. False discovery rate (FDR) was used to correct for multiple comparisons to control for the percentage of positives that are likely to be type one errors (Benjamini and Hochberg 1995).

Cross-sectional analyses.

In all analyses, LMERs were used to investigate the respective dependent variable with sex and treatment as fixed effects, and litter as a nested random intercept.

MRS. Individual metabolites were assessed with LMERs weighted by the "absolute standard deviation", a confidence measure of the concentration estimate. The "absolute standard deviation" is the standard deviation output from LCModel scaled to the concentration of the individual metabolite. False-discovery rate was used to correct for multiple comparisons across the mass univariate linear models across all metabolites.

OFT. Four ratios were calculated between the center and outside perimeter (edges/corners): duration of time spent, frequency of passes, velocity, and distance traveled. Ratios were assessed with LMERs.

Sociability. Sociability index was assessed for both time spent (duration) and frequency of passes with the following equation:

 doi:10.20944/preprints202203.0136.v1

$$[Intruder\ sniffing/(intrudersniffing + objectsniffing)] - 0.5$$

The 0.5 was subtracted from the resulting ratio to center the data such that positive values indicated more social time.

Novelty. Novelty index was assessed identically to the sociability index, where the social zone becomes the new intruder, and the object zone becomes the familiar intruder.

PPI. Prepulse inhibition was assessed as %PPI, calculated with the following formula: the percentage of PPI = 100 × (average startle trials - startle response for prepulse)/(average startle trials). A higher %PPI corresponds with the expected behavior (reduced startle) of wildtype mice. To analyze group or sex differences, LMERs were employed with the interaction between treatment and prepulse level and sex as fixed effects, mouse ID and litter as random effects, and %PPI as the dependent variable.

3. Results

3.1. Exposure to Poly I:C Alters Trajectories of Neurodevelopment

POL offspring, compared to SAL offspring displayed significant alterations in developmental trajectories in clusters of voxels throughout the brain ($t = 4.73$, <1% FDR). More specifically, volumes in the left CPu, right arbor vitae/Crus I exhibited a steeper increase in volume from PND35 and 60 in POL relative to SAL offspring. In contrast, a steeper decline in volume was observed in the anterior right CPu, left lateral septal nucleus, anterior commissure/hypothalamus, sensorimotor cortex, left Cornu Ammonis (CA)1 and CA3 of POL relative to SAL offspring from PND35 to 60 (visualized at 20% FDR Fig 2, at 5% FDR sFig 3). There was no main effect of sex, even at 20% FDR.

3.2. Exposure to Poly I:C Contributed to Subtle Changes to Brain Chemistry

Following quality control (excluded n=3), seventeen SAL mice (9 female, 8 males) and twenty POL mice (10 female, 10 male) were included for analysis. At PND 60, but not at PND 35, POL offspring had trending decreases in concentration of Ins ($p = 0.08$) and GABA ($p = 0.06$) in the anterior cingulate cortex (Fig 3), however no effects survived correction for multiple comparison, limiting their interpretation. Additionally, in POL offspring, there were trending decreases in concentration of creatine ($p = 0.09$), phosphocreatine (PCr) ($p = 0.08$), and N-acetyl aspartate ($p < 0.09$) (sFig 4).

3.3. Exposure to Poly I:C did not induce significant changes to behavior

During adolescence in the open field test, POL offspring demonstrated a trending decrease in the ratio of distance traveled in the center of the open-field box compared to the perimeter (edges/corners) ($p = 0.16$), however the ratio of velocity, frequency of passes, and duration was not different, nor were any metrics during adulthood (Fig 4).

In the social preference test, mice did not express a preference for the right or left chambers at baseline (sFig 5a and b). First we examined whether SAL offspring exhibited the expected preference for the social area (chamber, cage, and sniffing area) compared to the object area (chamber, cage, and sniffing area). The SAL offspring preferred the social area to the object area during adolescence ($p < 0.001$), but not during adulthood ($p = 0.33$). Therefore, we compared the SAL offspring to the POL offspring during adolescence but found no difference between the groups in either duration ($p = 0.84$) or frequency ($p = 0.82$) of ratios (sFig 5c and d). Following exclusion for video quality, data represent seventeen SAL offspring (10 females, 7 males) and eighteen POL offspring (9 females, 9 males).

In the novelty preference test, the SAL offspring did not exhibit the expected preference for the novel areas over the familiar areas (sFig 5e and f). In prepulse inhibition, the POL offspring did not demonstrate alterations in sensorimotor gating as measured with prepulse inhibition at either timepoint (sFig 6).

 doi:10.20944/preprints202203.0136.v1

4. Discussion

In this study we present multiple approaches to examining the impact of early prenatal exposure to POL between adolescence and early adulthood. Using longitudinal neuroimaging we provide whole-brain, voxelwise trajectories of brain volume change. To complement the analysis of structural neuroanatomy, we leveraged MRS to assess *in vivo* chemical changes in the ACA, a region previously shown to be affected by prenatal MIA-exposure, and commonly associated with neurodevelopmental disorders, as well as behavioral assessments relevant to neurodevelopmental pathology at two timepoints. Our findings contribute to the understanding of the impact of the POL MIA model in mice, further characterizing the effects on multiple modalities.

Contextualization of neuroanatomical findings

Overall, focal areas of significant volume change between treatments over time are evident throughout the brain, prohibiting deep discussion of each significant cluster of voxels, so interpretation is limited to voxels with the most significant change. The structures in which we observe volumetric changes are similar to those previously reported from our laboratory (Guma et al. 2021). While direct comparison between the directions of results (increase or decrease) are not possible because of methodological differences in the number of scan timepoints (two versus four) and the modeling (linear versus quadratic/cubic trajectories), close examination of the results in adolescence and early adulthood reveals that for some of the regions (subiculum, nucleus accumbens) the direction of volumetric changes are different, with Guma et al., reporting relative overshooting in POL growth and the present study finding relative decrease in volume. Other areas (cerebellar regions, striatum) are in alignment, with both studies reporting relative volume increases between those timepoints. Because the current experiment assessed only two timepoints, rather than four in order to expand into multiple modalities, it has a lower temporal resolution, potentially losing some of the changes only observed over a longer developmental period. Longitudinal MRI in rats following exposure to POL on GD 15 demonstrates altered trajectories of brain development reporting decreased volume in the hippocampus, striatum, and prefrontal cortex, and increased volume in the lateral ventricles, aligning with our findings in the right caudate putamen, and hippocampus, although we did not see increased ventricular volume (Piontkewitz et al. 2011; Crum et al. 2017).

Cross-sectional studies performed *ex-vivo* report no neuroanatomical differences surviving corrections for multiple comparisons, substantiating the subtlety of the results at a given timepoint and emphasizing the need for longitudinal studies in elucidating developmental differences, both in rodents, and potentially in humans (Mueller et al. 2020).

Between the adolescent and early adult time points, two areas prominently showed increased volume in the POL group compared to the SAL: the left CPu and right arbor vitae/Crus I of the cerebellum. The CPu constitutes the dorsal striatum (Szczypka et al. 2001), and in mice, unlike in humans, it represents a single undifferentiated structure responsible for integrating information involved in motor control, emotion, and cognition (Schröder, Moser, and Huggenberger 2020). Increased volume and altered shape were reported in the caudate and putamen of individuals with chronic schizophrenia (Mamah et al. 2007), and trends towards larger caudate volumes have also been observed in first episode psychosis (FEP) (Cuesta et al. 2017) and early onset psychosis (Gurholt et al. 2022). Nevertheless, the literature is mixed, with some meta-analyses finding no volume differences in caudate or putamen in individuals with schizophrenia (van Erp et al. 2016), while others demonstrate increased volume in both structures (Okada et al. 2016). One source of variability in human studies may be the impact of antipsychotics, as typical neuroleptic treatments have been associated with higher caudate volumes as well (Scherk and Falkai 2006). Compared with typically developing children, those with a diagnosis of ASD also demonstrated increased caudate and putamen volumes, however these differences do not survive when controlling for cerebral volume (Estes et al. 2011). Interestingly, our results

 doi:10.20944/preprints202203.0136.v1

reflect the bidirectional reports in the literature, with focal increased volume in the left CPu, but decreased volume in a more anterior region of the right CPu. Crus I in mice has been identified as homologous to Crus I and II in humans, responsible for cognitive and visuomotor function (Sugihara, 2018). Meta-analyses and voxel-based morphometry studies reveal increased volume of Crus I in patients with schizophrenia (Ding et al. 2019; Kuhn et al. 2012).

The majority of regions were associated with volume decrease in POL versus SAL, including the left subiculum, left lateral septal nucleus, anterior commissure/hypothalamus, and left CA1/CA3 and right CPu. The septal nuclei have previously been implicated in the pathophysiology of neurodevelopmental, affective disorders (Brisch et al. 2011). Previous evidence implicates the hypothalamic-pituitary-adrenal axis and stress reactivity in the robust reports of decreased hippocampal volumes found in FEP and schizophrenia (Pruessner et al. 2015; Adriano, Caltagirone, and Spalletta 2012; Nelson et al. 1998; Pantelis et al. 2003; Steen et al. 2006; Velakoulis et al. 2006). Further investigation of this relationship may be of special interest as the hypothalamic nuclei were areas of greatest alteration in our study. As the overall impact of MIA on the behavioral phenotype is subtle, this study could draw attention to regions implicated in neurodevelopmental disorders that are most vulnerable to prenatal immune insults, as they show early volumetric changes.

Minimal Impact of MIA on ACA Neurochemistry

GABA, Ins, NAA, Cr, and PCr show slight trending reductions in concentration at the second timepoint. Offspring of dams exposed to POL on GD 12.5 and 17.5 exhibit GABAergic malfunctions in the ACA in adolescence accompanied by hyperexcitability and decreased sociability (Okamoto et al. 2018). While subtle, reductions of GABA concentrations could reflect changes to the excitatory/inhibitory balance. Rat offspring of dams exposed to lipopolysaccharide on GD fifteen and sixteen demonstrated reductions in Cr, but increases in PCr in *ex-vivo* MRS acquired from tissue in the cortex and striata (Capellán et al. 2019). Changes in creatine are especially important as the peak is often used as a reference for estimates of other metabolite concentrations, but there is evidence it is altered in affective disorders, and it serves as a marker for cellular energy production (Öngür et al. 2009).

Myo-inositol is a marker of microglial cells and the elevation of this metabolite is often interpreted as activation of the brain's innate immune system (Plitman et al. 2016). In the human literature, increases in Ins have been reported in FEP in conjunction with altered glutamate concentrations (Plitman et al. 2016). Other studies investigating MRS in the temporal lobe find increased levels of Ins in chronic schizophrenia, but not FEP, suggesting the metabolic changes may arise later (Wood et al. 2008). In contrast, the reduction of Ins in POL offspring is in line with our hypotheses and previous rodent MIA literature (Li et al. 2015), implying the immune system may be impacted long-term, not only in gestation. Ins has been viewed as a mark of astrocytes, and reduction in this metabolite may indicate disruptions in astrocytes that could contribute to psychiatric disorders (Webster et al. 2005). Contrary to previous studies, however, we did not see alterations to NAA. An important methodological difference between our analyses and similar previous studies is our analysis of the absolute metabolites with concentrations estimated relative to the water peak, rather than "relative" metabolites considered relative to the Cr peak, as is often seen in MRS studies. As our study indicated trending differences in Cr levels ($p = 0.09$) it would be inappropriate to study the "relative" metabolite concentrations, but this could account for differences in results compared to the prior literature (Li et al. 2015). Unaltered NAA may indicate that neuronal density and function is not significantly altered at the observed timepoints, consistent with the lack of difference in behavioral results.

Parallels with Humans and Nonhuman Primates

A recent longitudinal MRI study in rhesus monkeys revealed POL injected at the end of the first trimester showed reductions in gray matter volume in the prefrontal and frontal cortices between 6 and 45 postnatal months (Vlasova et al. 2021), comparable with results in our model. Discrepancies between results could be due in part to differences in the induction of MIA (immunostimulatory agent, dose, gestational timing) (Kentner et al. 2019).

Although epidemiological evidence cannot establish a causal link between MIA and neurodevelopmental disorders, they provide an important link between MIA experiments in animal models and disorders in humans (Estes and McAllister 2016). While translation between species can be especially difficult, MRI presents a noninvasive method that can be employed to study neurodevelopment across species (Guma et al. 2019; Chakravarty and Guma 2021). This study spans the homologous time period where risk is greatest for neurodevelopmental disorders, such as psychoses, to emerge in humans (Hare et al. 2010; Guma, Plitman, and Chakravarty 2019). As discussed in previous sections, many of the regions altered in POL offspring are also implicated in human studies of neurodevelopmental disorders. Nevertheless, the association to the human condition should be made with caution, especially given the imperfect translation between brain regions in the mouse and human and imperfect homology between developmental epochs (Schröder, Moser, and Huggenberger 2020).

Limitations

The results for this paper should be considered in light of the limitations in our experimental design. As previously mentioned, our study was primarily powered to examine MRI-derived differences at the level of structures and not for the MRS and behavioral tests used. This is due, in part, to the lack of homology and standardization across these latter two assays in the literature, making it difficult to develop a useful power estimation. Nevertheless, many studies do include comparable sample sizes for similar behavioral tests (Bitanihirwe et al. 2010; Gibney et al. 2013). Secondly, while 2 timepoints are enough to establish trajectories of brain volume growth, increasing the number of timepoints would allow more nuanced understanding of alterations to the developmental trajectories, as we have shown in our previous work (Guma et al. 2021; Kong et al. 2018; Rollins et al. 2019).

One source of variation in the literature could also be differential severity in the dams' inflammatory responses, with POL eliciting an increased cytokine profile in some dams compared to others (Mueller et al. 2020). In our experiment, the cytokine response to POL was measured in a separate subset of dams at GD 9, as the trunk blood collection for the cytokine panel was a terminal procedure (SM 1.1). While the data verified that POL activated the maternal immune system, the subset of dams did display varied responses, suggesting milder responses in the immune activation may underlie the variability observed in brain and behavior outcomes.

These limitations provide guidance for future directions in MIA research. Sources of variability in the literature could be due not only to experimental differences outlined previously (Mueller et al. 2018; Kentner et al. 2019), but also variance in timing of assessment of outcome measures (early life, adolescence, or adulthood), and even number of timepoints of assessment (cross-sectional vs two or more timepoints), leading to great variability across the literature (Vlasova et al. 2021; Bauman et al. 2013; Garay et al. 2013; Kreitz et al. 2020; Guma et al. 2021; Ronovsky et al. 2017; Yee et al. 2011; Vigli et al. 2020; Kowash et al. 2019; Mueller et al. 2020).

While many studies investigate the impact of MIA on neurodevelopment, one main advantage of rodent studies is the experimental nature allowing researchers to draw causal relationships between the MIA and resulting changes. New methods allow researchers to expand the characterization of the effects of MIA on offspring, and future studies can leverage experimental manipulations to investigate the interaction between different risk factors such as genetic predisposition, additional gestational exposures

(such as maternal stress), or adolescent exposures (such as cannabis exposure) (Yee et al. 2011; Ranaei et al. 2020; Rymut et al. 2020). Future studies could also leverage multivariate approaches, such as partial least squares regression, to investigate patterns of variability across different modalities.

5.Conclusions

The present study serves the literature in two ways. First it provides evidence of the subtle neuroanatomical changes that MIA exposure in early gestation can have on structural brain development, in conjunction with neurochemical and behavioral phenotypes. Second, it provides suggestions for future studies that strive to further elucidate these impacts, as discussed above. Future studies ought to carefully consider experimental design relating to sample size and the variability of outcome measures. A more nuanced appreciation of the long term outcomes of MIA early in pregnancy may help direct attention in consideration of the intervention and ultimately prevention of neurodevelopmental disorders.

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