Maternal Immune Activation as a Risk Factor for Neurodevelopmental Disorder: Phenotyping Mouse Offspring

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Neurodevelopmental disorders are a heterogenous class of conditions including schizophrenia and autism spectrum disorder (ASD) which present with a variety of deficits in social, cognitive, physical, and language domains [1, 2]. Schizophrenia is characterized by positive symptoms such as hallucinations and delusions, negative symptoms such as anhedonia and lack of motivation, and cognitive symptoms such as deficits in working memory and behavioural flexibility [3, 4, 5]. ASD is characterized by social deficits as well as repetitive and restricted behavioural patterns and interests [1, 6]. Individuals living with neurodevelopmental disorders can experience significant burden, and this burden extends both financially and emotionally to family members, care-givers, and society [7, 8].

Disorders such as schizophrenia and ASD are thought to emerge as the result of a combination of factors which, in concert, manifest as altered trajectories of brain development [3, 9, 10, 11]. In particular, activation of the maternal immune system during pregnancy has been identified as a risk factor for several neurodevelopmental disorders in the offspring [10, 12, 13]. While some maternal immune activation (MIA) research has been conducted in humans, this approach is generally limited to epidemiological studies [10]. Thus, preclinical animal research has emerged as an effective method to study the effects of MIA in rodents.

While rodent studies have provided evidence for structural brain abnormalities and behavioural deficits in adult offspring associated with MIA [14, 15], there is currently a gap in our understanding of MIA effects in early postnatal life. The research I performed this past academic year, under the supervision of Elisa Guma and Dr. Mallar Chakravarty, addressed this missing knowledge in MIA rodent literature. My research investigated the effects of MIA induced on gestational day (GD) 9 (the late first trimester of pregnancy [16]) on mouse offspring at postnatal day (PND) 8. The impact of MIA was quantified behaviourally with measures of ultrasonic vocalization (USV) from offspring (n = 41) when separated from their mother, which reflect attempts to elicit maternal attention and are used to assess communicative ability [17]. Neuroanatomical details were investigated using structural magnetic resonance imaging (MRI) to assess the relative volumes of brain structures (n = 16); MRI allows for whole-brain analysis using a technique translatable for human research.

Two groups of mice were studied: healthy control offspring born to a dam injected with saline (the SAL group), and MIA-exposed offspring born to a dam injected with polyinosinic-polycytidilic acid (poly(I:C)), a synthetic viral mimetic which induces an acute immune response (the POL group), at GD 9. On PND 8, the USV task was administered and animals were sacrificed via intra-cardiac perfusion with paraformaldehyde and Gadolinium (an MRI contrast

agent). Brains of the animals were scanned *ex vivo* using a Bruker BioSpec 7T animal scanner to acquire T2-weighted structural MRI at 70 μm isotropic resolution.

USV data showed no significant difference between group means – however, the POL group showed significantly greater variance compared to the SAL group for several USV metrics.

The behavioural USV results potentially point to MIA as a *risk factor* for neurodevelopmental disorders – it does not affect individuals within a group uniformly, which would be seen as a shift in the value of the group mean. Rather, one might expect isolated risk factors to cause changes in specific individuals, forming clusters outside the range seen in a sample of healthy controls and increasing variance. The onset of disorders such as ASD or schizophrenia may result from multiple "hits" from different risk factors or susceptibility factors combined in an individual, rather than a single isolated factor such as MIA [10, 11].

Analysis of structural MRI data revealed significant relative volumetric decreases in the cerebellum, medial orbitofrontal cortex (mOFC), left nucleus accumbens (NAc), and left hippocampus; relative volumetric increases in the left ventromedial thalamus and left corpus callosum. These brain regions have consistently been reported to undergo altered development in individuals suffering from neurodevelopmental disorders.

For example, dysfunction of a cortico-thalamic-cerebellar circuit has been proposed to underlie negative and cognitive symptoms in schizophrenia, and reduced cerebellar volume has been associated with both ASD and schizophrenia [18, 19]. The decreased volume in the mOFC parallels abnormal findings with human schizophrenic patients – hypoactivation and decreased volume of the OFC have been associated with impairments in decision-making, rule-learning, and ordered thinking, and abnormalities in this region have been proposed to mediate ASD symptoms such as social impairment and repetitive behaviour [20, 21, 22]. In addition, decreased hippocampal volume is consistently reported in human schizophrenia studies [23]. A hypothesis of dysfunctional dopamine signalling has been proposed as a pathological mechanism involving structures within the mesolimbic circuit, where hyperactive hippocampal activity gated by the NAc is thought to drive abnormal dopamine activity [24].

In summary, this project contributes to our understanding of how complications during pregnancies may critically alter brain development trajectories and thereby elevate risk for neurodevelopmental disorders. Further, this research improves our understanding of MIA and its behavioural and neural underpinnings. The structural brain differences between the POL and SAL groups at PND 8 are particularly interesting when considering the lack of group differences but increased variability in USV behaviour. These results imply that the MIA risk factor may provoke abnormalities in brain structure which may in turn lead to disrupted behaviour in *certain* individuals who are more susceptible to the effects of these structural aberrations. Essentially, disrupted structural neurodevelopment does not necessitate changes in behaviour in all offspring – rather, MIA appears to increase the variance in the affected group's behaviour. Future studies may consider parsing this heterogeneity to better understand the factors underlying risk and resilience. These findings suggest that it may be possible to view a single risk factor's differential impact on brain and behaviour – although significant structural differences can be

observed at the group level, this in and of itself does not link to the emergence of altered behaviours. Information gathered from preclinical animal models can guide efforts in clinical human research – a deeper understanding of the etiology of neurodevelopmental disorders can contribute to the development of more personalized treatments with higher efficacy as well as preventative strategies.

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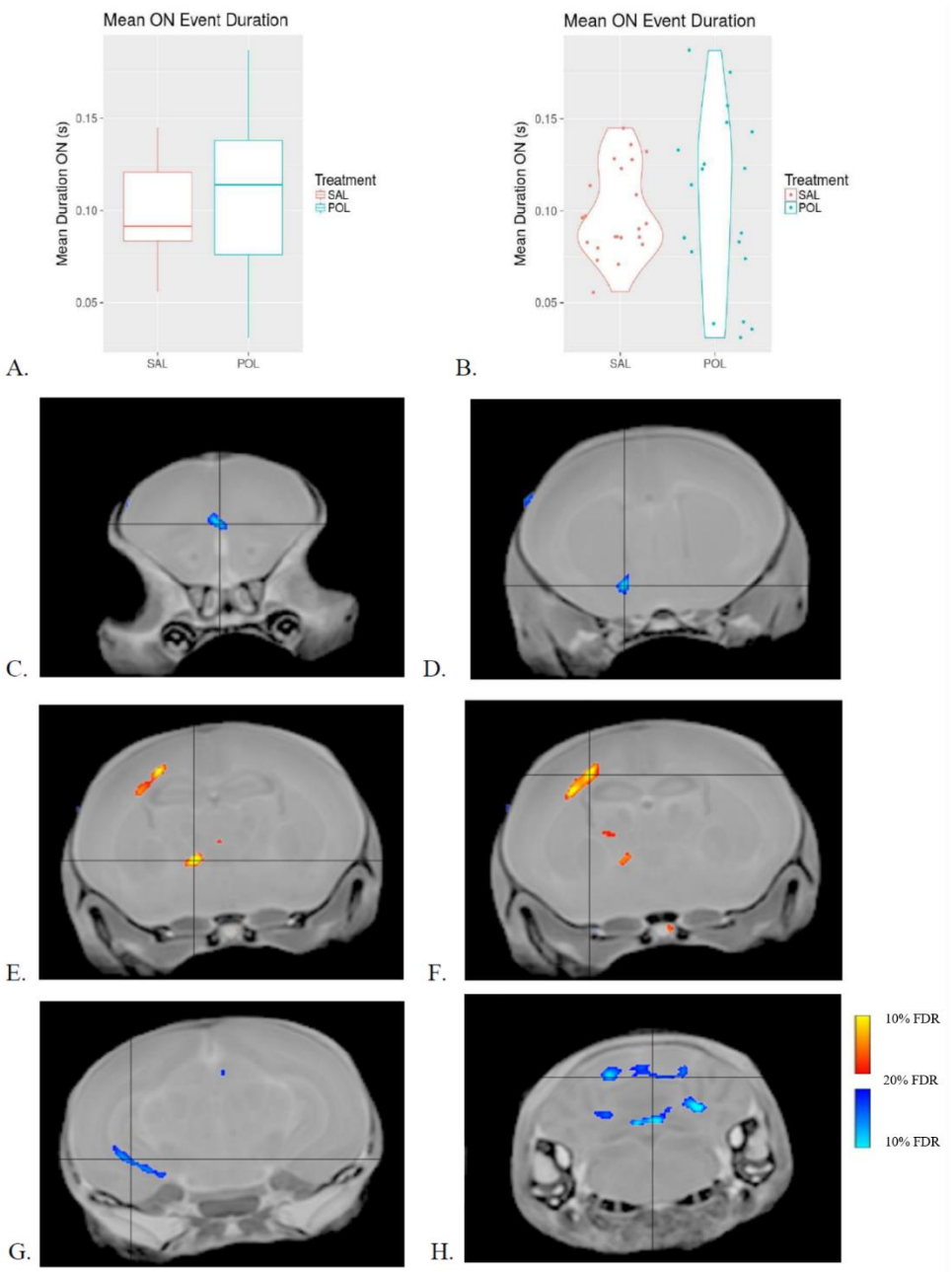

Figure 1. A) USV data for the metric of Mean ON Event Duration (the average duration of offspring vocalization), separated by treatment (SAL vs POL). **A)** shows a traditional boxplot and **B)** shows a violin plot, emphasizing the differing distributions of each group with individual data points plotted. Mean ON Event Duration does not differ significantly between groups ($p = 0.734 > 0.05$). However, the variances of the two groups are significantly different [$F(18, 21) = 1.43$, $p = 0.003$]. Significant relative volumetric decreases were observed in the mOFC **(C)**, left NAc **(D)**, left hippocampus **(G)**, and cerebellum **(H)** in the POL group compared to the SAL. Significant relative volumetric increases were observed in the left ventromedial thalamus **(E)** and left corpus callosum **(F)** in the POL group compared to the SAL. These differences are significant when corrected for multiple comparisons using the False Discovery Rate (FDR), where the light blue in this figure indicates relative volumetric decreases significant at 10% FDR, and the dark blue indicates decreases significant at 20% FDR; the yellow indicates relative volumetric increases significant at 10% FDR and the red indicates increases significant at 20% FDR.