Parvalbumin and perineuronal net expression in the medial and lateral regions of the dorsal striatum in an idiopathic model of ASD, through development and between sexes.

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1 INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterised by (1) social/communication deficits and (2) restrictive, repetitive behaviours, interests, and activities (RRBIs).

The **striatum** is a key inhibitory nucleus of the basal ganglia, noted to facilitate motor behaviours and action selection mechanisms such as reward-motivated behaviours and behavioural flexibility. Impairment of action selection mechanisms may underlie RRBI expression in ASD.

Parvalbumin-expressing fast-spiking interneurons (PV+FSIs) are suggested to influence striatal output in both the direct and indirect pathways by modulating medium spiny neuron signalling. The fast-spiking activity of PV+FSIs is supported by **perineuronal nets (PNNs)** that preferentially encapsulate these neurons.

Altered expression of PV+FSIs was reported in both post-mortem investigations of ASD and in the striatum of a variety of genetic and environmental models of ASD. Altered striatal PNN expression has also been identified, with digestion of PNNs reported to reduce repetitive behaviour in the idiopathic BTBR model of ASD. However, prior investigations tend to utilise models at only one developmental stage despite the dynamic nature of ASD through development.

Altered PV+FSI expression in the striatum may contribute to RRBI expression in ASD, supported by PV+FSI ablation studies where a ~40% reduction in striatal PV+FSIs resulted in increased motor stereotypy in male mice; these behavioural changes were not observed in female mice. Few studies have investigated if altered striatal PV+FSI expression in ASD occurs uniformly or in a subregionally dependent manner.

The aim of this study was to use immunohistochemical techniques to investigate differences in the density of PV+FSIs and their associated PNNs in an idiopathic model of ASD (BTBR mice) relative to typical development, within the dorsomedial striatum (DMS) and dorsolateral striatum (DLS) at two developmental stages (3-4wk and 6-8wk), in both sexes.

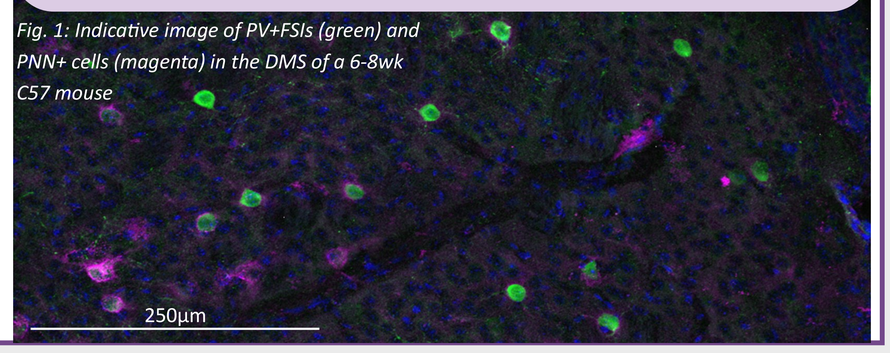

Fig. 1: Indicative image of PV+FSIs (green) and PNN+ cells (magenta) in the DMS of a 6-8wk C57 mouse

2 METHODOLOGY

The expression of PV+FSIs and associated PNNs was investigated via immunohistochemical (IHC) analysis. Fixed tissue from 40 mice was used to investigate differences between strain (BTBR T+ Itpr3tf/J (BTBR), C57 L/J (C57)), sex (Male, Female), developmental stage (3-4wk, 6-8wk), and striatal subregion (DMS, DLS), as shown in Table 1.

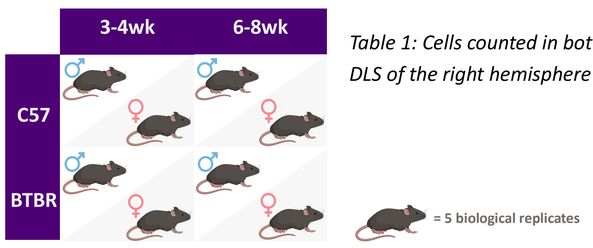

Table 1: Cells counted in both the DMS and DLS of the right hemisphere of each mouse.

Five coronal slices (25µm) within the bregma range 1.10mm to -0.10mm were selected for IHC analysis (with reference to the Paxinos & Franklin (2007) mouse brain atlas; Fig. 2.1).

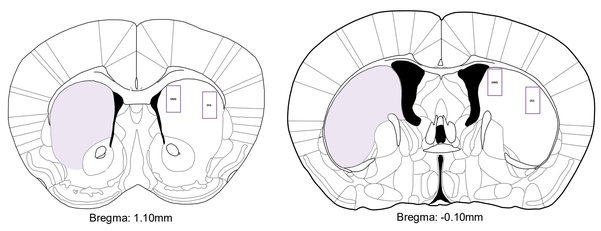

Fig. 2.1: Coronal sections of mouse brain at bregma 1.10mm and –0.10mm, indicating the anterior and posterior limits of this bregma range, the DMS and DLS ROI are indicated. Adapted from the Paxinos & Franklin (2007) mouse brain atlas.

Sections were stained for PV (green) and PNNs (red), with DAPI stained nuclei (blue; Fig. 2.2). Counting was performed in FIJI on tiled z-stack images at x20 magnification, with the mean density of cells calculated from the mean of all five sections.

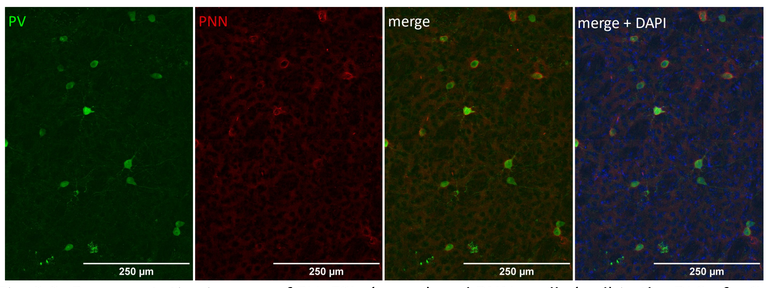

Fig. 2.2: Representative images of PV+FSIs (green) and PNN+ cells (red) in the DLS of a 6-8wk male C57 mouse. Slices were also counterstained with DAPI (blue). Scale bar: 250µm

4-way mixed ANOVAs were performed in R to analyse collected data (square-root transformed PV+PNN+FSI density values).

3 KEY RESULTS

Significantly lower density of PV+FSIs and PV+PNN+FSIs within the DMS of BTBR relative to C57 mice.

- But a greater density of PV+FSIs in the DLS of BTBR relative to C57 mice.
- See Fig. 3.1 for PV+FSI density, and Fig. 3.2 for PV+PNN+FSI density

Significantly lower density of PV+FSIs and PV+PNN+FSIs within the DS through development of C57 but not BTBR mice.

- Between developmental stages of 3-4wk and 6-8wk.
- See Fig. 3.3 for PV+FSI density, and Fig. 3.4 for PV+PNN+FSI density

Significantly greater density of PV+FSIs within the DMS of male relative to female mice.

- Further, a significantly greater basal density of PV+FSIs and PV+PNN+FSIs within the DLS of female relative to male mice.
- See Fig. 3.1 for PV+FSI density, and Fig. 3.2 for PV+PNN+FSI density

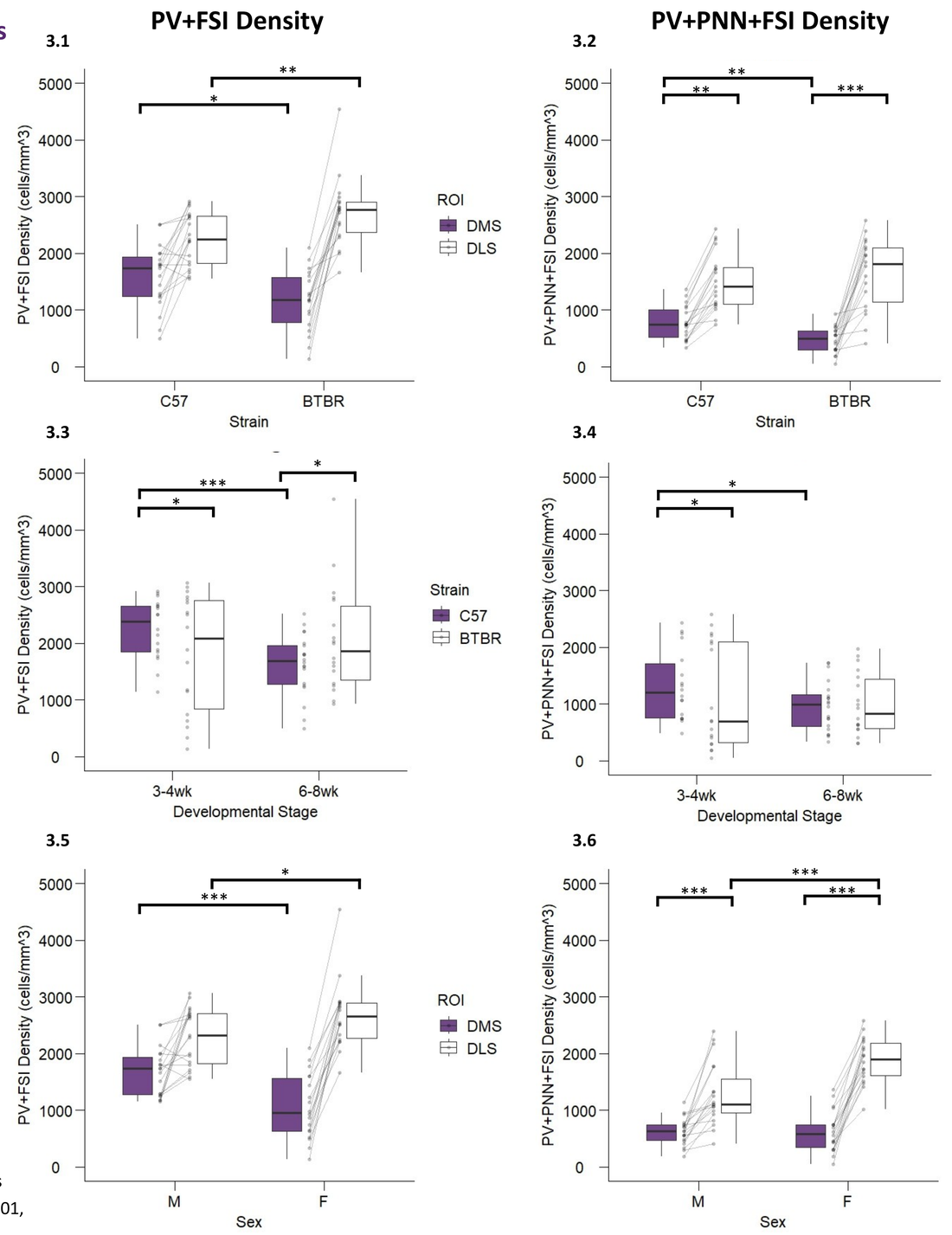

Fig. 3. Boxplots displaying the mean (±SD) density of PV+FSIs and PV+PNN+FSIs per mm^3 in the DS. Small dots represent individual datapoints. * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$

4 DISCUSSION AND FUTURE DIRECTIONS

Reduced PV+PNN+FSI density in the DS of BTBR may largely be explained by reductions in the DMS

No significant differences in the relative fluorescent intensity of PV staining, and the relative expression of pvalb mRNA, were identified between C57 and BTBR mice (data not shown). Therefore this reduction in PV+PNN+FSI density in BTBR mice is likely to represent cell loss rather than just reduced protein expression. DS subregion-specific alterations in ASD may be key to consider whilst investigating ASD aetiology due to the related but functionally distinct contributions of these regions to action selection mechanisms and repetitive behaviour.

Observed reductions in cell density through development not present in BTBR mice

The lack of PV+FSI density reductions through development in BTBR mice, whilst present in C57 mice may highlight another mechanism by which development is altered in the striatum in ASD, which may align with the persistence of RRBIs beyond developmentally appropriate stages in ASD.

Sexually-dimorphic densities of PV+FSIs in the DMS and DLS

A greater density of PV+FSIs was observed in male relative to female mice in the DMS, whilst a greater density of PV+FSIs was observed in female relative to male mice in the DLS. This sex difference may emphasise the importance of considering sex in conditions such as ASD where RRBIs are expressed, suggesting the involvement of these neurons in wider circuits may differ (though further experimentation is required). This perspective may partially explain why prior medial DS PV+FSI ablation studies elicit RRBIs in male but not female mice.

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