

Identification of extracellular matrix plasticity during tactile sensation in a female mouse model of Rett Syndrome

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DEDICATION

For my grandmother and uncle, who were always so supportive of this massive goal but were not able see me complete it.

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No PhD is earned alone, but that is particularly true of this one.

ABSTRACT

Rett Syndrome (RTT) is a neurological disorder caused by mutations in *MECP2*, a gene expression regulator. The emerging hypothesis of RTT pathogenesis is that RTT is a sensory processing disorder which causes the onset of higher-order cognitive and social communication deficits seen in syndromic patients. Using a female *Mecp2*-heterozygous (Het) mouse model of RTT, our lab showed *MECP2* is essential for regulating experience-dependent plasticity, measured by perineuronal net (PNN) expression in the adult auditory cortex during the pup gathering paradigm. PNNs are extracellular matrix structures that form around cortical inhibitory interneurons solidifying synaptic connections made after new experiences. As the pup gathering paradigm is multisensory, we chose to investigate how *MECP2* regulates the timing of experience-dependent plasticity within another sensory modality important to the behavior: somatosensation. Through whole brain manual quantification of PNN expression within the primary somatosensory cortex (SS1) in the adult Het brains before (Naïve) and after (Sur) pup gathering behavior, we found an increase in PNN density in the Naïve and SurHet SS1. Atypical increase of PNN density within the NaïveHet SS1 suggested adult Hets are unable to process tactile information causing inefficient pup retrieval. We confirmed that adult Hets have deficits in tactile perception through simple whisker-based tactile tasks (Tac). The adult atypical NaïveHet PNN expression in SS1 is not present between 6-week-old (WO) NaïveHet and WT females, suggesting these animals would not present with the same tactile phenotypes as the adults. 6 WO Hets do not present with tactile perception deficits but are inefficient at the pup gathering behavior. Whole brain quantification of PNN density in the 6 WO Tac brains showed little significant alterations in PNN density between the TacHet and TacWT SS1. Comparatively, we found a significant increase in SurHet SS1 PNN density compared to SurWT and NaïveHet, which was present in all subregions. Together, these data suggest that *MECP2* deficiency affects experience-dependent plasticity, as measured by PNN expression, during complex multisensory but not simple tactile behaviors within the SS1, in an age-dependent manner.

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INTRODUCTION

Rett Syndrome (RTT) is a neurological disorder characterized by the onset of motor, social communication, and higher-order cognitive deficits after a period of typical development, at around two years of age (Chahrour and Zoghbi, 2007). More recent studies have shown that RTT patients exhibit subtle sensory processing deficits during the period of 'typical' development, suggesting that RTT is a sensory processing disorder, which gives rise to the syndromic characteristics later in development (Buchanan et al., 2019; Djukic and Valicenti McDermott, 2012; Neul et al., 2014; Nomura, 2005). RTT is caused by mutations in methyl CpG-binding protein 2, *MECP2*, which is an activity-dependent gene expression regulator (Amir et al., 1999). As *MECP2* is an X-linked gene, most male patients are missing their single copy of the gene and die in infancy while female patients have mosaic (heterozygous) expression of functional MECP2 protein (Amir et al., 1999; Chahrour and Zoghbi, 2007). MECP2 has been shown to regulate over 3000 different genetic targets in response to neural activity and experience throughout development and adulthood (Cohen et al., 2011b). However, the underlying pathology of how mutations in *MECP2* cause these neurological symptoms is unknown. Based on what is known about MECP2 function and timing of RTT symptomatic onset, the current hypothesis is that MECP2 regulates experience-dependent plasticity mechanisms during periods of heightened sensory experience in a context-dependent manner. MECP2 deficiency causes visual (Krishnan et al., 2015), sensorimotor (Morello et al., 2018), and auditory (Krishnan et al., 2017; Lau et al., 2020) sensory processing deficits in the *Mecp2*-null male and *Mecp2*-heterozygous female mouse models of RTT (Krishnan et al., 2015, 2017). In the visual and auditory cortex of these models, dysregulation of the inhibitory network coincided with the precocious or atypical formation of perineuronal nets, extracellular markers of restricted plasticity, showing that this was a common motif in both development and adulthood. This dissertation identifies another context in which MECP2 deficiency causes sensory deficits, this time in tactile processing, and supports that dysregulation of perineuronal net formation during experience-dependent events is a common motif in RTT pathogenesis in the clinically relevant mouse model.

Rett Syndrome Characteristics

RTT Syndromic Phenotypes in Patients

RTT is generally characterized as a neurodevelopmental disorder, as the appearance of syndromic phenotypes occurs after a period of, seemingly, typical development; for this reason, RTT is mostly studied in a developmental context. The general characteristic symptoms of RTT are the development of hand wringing stereotypies, loss of fine and gross motor movement, and social communication deficits (Figure 1, Gray) (Chahrour et al., 2008; Hagberg et al., 1983). Recent studies show that these syndromic features are preceded by subtle deficits in sensory processing, which contribute to the development of more characteristic symptoms of the disorder (Figure 1, Green) (Buchanan et al., 2019; Djukic and Valicenti McDermott, 2012; Nomura, 2005). RTT patients can live into late adulthood and the syndromic symptoms persist throughout their life, often getting progressively worse, though this stage is not as well studied.

The Natural History of Rett surveys show that there were subtle changes in sensory processing in RTT children which precede the general phenotypic onset that was first described by Andreas Rett in 1966 then by Bengt Hagberg in 1983 (Hagberg et al., 1983; Rett, 1966). The time before gross symptomatic onset (around 2 years of age) was seen as a 'pre-symptomatic period', which was recently found to not be the case – however that is how it will be referred to here, in adherence with the current literature as to separate the two phases of syndromic pathology: the period of subtle sensory deficits (3 – 24 months of age) and the onset of syndromic symptoms (after 24 months of age). The natural history study found that, while many RTT patients were able to start crawling, walking, talking (even learning some words) during this 'pre-symptomatic period', the acquisition of these skills was still delayed when compared to typically developing children (Neul et al., 2014). Other recorded symptoms within the 'pre-symptomatic period' include lack of anticipatory movement, social smiling and eye-to-eye contact, hypersensitivity to sound and sensory feedback (Figure 1, Green) (Buchanan et al., 2019; Djukic and Valicenti McDermott, 2012; Nomura, 2005). The recent characterization of subtle sensory deficits, in the 'pre-symptomatic' stage

suggest that these deficits are contributing to the higher-order cognitive and social communication deficits which are seen at symptomatic onset along with motor deficits (Figure 1, Magenta).

At around 2 years of age, patients develop motor stereotypies, specifically repetitive hand wringing motions (Figure 1, Magenta) (FitzGerald et al., 1990; Nomura, 2005). As they age, motor impairments worsen with muscular hypertonia resulting in the loss of fine and gross motor movement, such as loss in ability to walk, which coincides with breathing abnormalities (Figure 1, Magenta) (FitzGerald et al., 1990; Killian et al., 2017; Motil et al., 2012; Tarquinio et al., 2017, 2018). Neurological symptoms, such as seizures, loss in vocal communication and behavioral issues appear and worsen after the 2-year mark (Buchanan et al., 2019; Jian et al., 2006; Tarquinio et al., 2017; Vignoli et al., 2010). Patient symptoms can worsen over time, with seizures worsening and the development of Parkinsonian features in adulthood (Humphreys and Barrowman, 2016).

While there are many syndromic characteristics, diagnosis of the disorder can be difficult because not all RTT patients present with every symptom. The severity of RTT syndromic phenotypes vary between patients due to random X inactivation (in females) as well as the severity of *MECP2* mutation (females and males). Females with this disorder have one wild-type copy and one mutated copy of *MECP2*. Each cell only requires one of those X chromosomes to be active, and this process is selected at random resulting in mosaic expression of functional MECP2 protein within the body (Ribeiro and MacDonald, 2020; Wu et al., 2014). While this process would ideally result in a 50/50 ratio of cells expressing the functional copy of MECP2 to cells expressing the mutated copy, that is not always the case (Renthal et al., 2018). Studies in human patients and the female *Mecp2*-heterozygous mouse model have shown that phenotypic severity increases when there is a lower percentage of cells expressing the functional MECP2 in the subject (Dayer et al., 2007; Krishnan et al., 2017; Xinhua Bao et al., 2008).

In 1999, Amir et al. found that mutations in the gene *MECP2* were causing RTT (Amir et al., 1999). A mouse model of RTT was developed two years later by Guy et al

using the Cre-loxP system to remove exons 3 and 4 from the *Mecp2* gene, resulting in no functional MECP2 protein expression in the null males (*Mecp2*^{-y}) and mosaic protein expression in the heterozygous females (*Mecp2*^{+/-}). This line is perpetuated by breeding the heterozygous (Het) female with a WT male and the offspring is roughly 1:1:1:1 (*Mecp2*^{+y}: *Mecp2*^{-y}: *Mecp2*^{+/+}: *Mecp2*^{+/-}) (Guy et al., 2001). Both the male and female mouse model exhibited delayed onset of neurological RTT symptoms; similar to the patient population (Guy et al., 2001).

Phenotypes in RTT Mouse Model

Researchers most often use the *Mecp2*-null male mice to study RTT because the model has comparatively less phenotypic variation than the female Het model, due to its mosaic expression of the MECP2 protein. While both models exhibit delayed onset of neurological symptoms, the onset for males is around 3 weeks of age whereas the females are closer to 16 weeks of age (Guy et al., 2001). After onset, the health of the null males rapidly deteriorates, and they die around 6-8 weeks of age (adolescence). Rapid deterioration after onset is not seen in the females who can live 1-2 years (Guy et al., 2001). While the male model can be a simpler model to study the effects of MECP2 depletion, the drawback is that they do not survive to adulthood like the female Hets. The Het mouse more accurately models the female patient population, which has mosaic expression of wild-type MECP2 and lives into adulthood.

Before the appearance of syndromic phenotypes in the Het female mouse model there is evidence of deficits in sensory processing, just like in the human patients. These show up early in development, around 3 weeks of age, specifically in the sensorimotor reflexes (Figure 2, Green) (Picker et al., 2006; Santos et al., 2007). After weaning age, subtle deficits in social communication (auditory processing) and visual perception (binocular matching) are seen in the female and male RTT models, respectively (Figure 2, Green) (Krishnan et al., 2015, 2017; Liao et al., 2012). In females, the appearance of uncoordinated gait phenotype subtly begins around 12 weeks of age (Robinson et al., 2013) which is followed by hindlimb clasping around 16 weeks of age (Samaco et al., 2013; Stearns et al., 2007). In the null males, motor

deficits are seen at 3 weeks of age and present as hindlimb clasping, irregular breathing, and the progressive worsening of gait deficiencies that ultimately result in decreased movement (Guy et al., 2001; Santos et al., 2007; Stearns et al., 2007). The delayed onset of motor and social communication deficits, as well as the early onset of sensory processing deficits seen in the patients are also seen in the mouse models.

MECP2

Methyl CpG-binding protein 2 (MECP2) is a methylation reader that binds to DNA in an activity-dependent manner which has been implicated in many cellular functions, including transcription activation and repression, alternative splicing, and chromatin remodeling (Ebert et al., 2013; Lyst and Bird, 2015; Skene et al., 2010). MECP2 contains a methyl-binding domain, which recognizes methylated DNA, as well as a transcriptional repressor complex binding domain, which is intrinsically disordered (Amir et al., 1999). The transcriptional repressor complex binding domain can recruit gene expression activator complexes, repressor complexes and alternative splicing complexes to enhancers and promoters depending on the phosphorylated residues in that domain (Cohen et al., 2011b; Ebert et al., 2013; Zhou et al., 2006). The majority of RTT causing mutations occur within these domains, causing there to be no functional MECP2 or the expression of a partially functioning MECP2 protein (Amir et al., 1999; Brown et al., 2016; Neul et al., 2008). For example, the missense mutation T308A in the transcriptional repressor complex binding domain prevents activity-dependent phosphorylation at this residue inhibiting the binding of the co-repressor complex NCoR to MECP2 (Ebert et al., 2013).

Variability in phenotypic severity can often be traced back to the location and type of mutation in the *MECP2* gene. There are over 300 known RTT-causing mutations within the patient population resulting in amorphic and hypomorphic alleles (Brown et al., 2016; Neul et al., 2008). RTT patients with amorphic alleles tend to have earlier onset of and more severe syndromic phenotypes, as these cells produce no functional MECP2 protein. However, those with hypomorphic alleles still make a less efficient MECP2 protein which, depending on the domain and the residue that was

modified, can result in milder symptoms (Neul et al., 2008; Villard, 2007). Male patients, who only have one X chromosome, have hypomorphic alleles, as those that have null alleles normally do not survive past their first year (Masuyama et al., 2005; Neul et al., 2018).

MECP2 in neurons

MECP2 is expressed almost universally across tissue types but is most highly expressed in neuronal tissue where it has been recorded as regulating over 3000 different genetic targets (Cohen et al., 2011b; Jung et al., 2003; Pelka et al., 2005; Song et al., 2014). MECP2 is broadly expressed throughout the brain during development and adulthood (Kishi and Macklis, 2004; Metcalf et al., 2006; Pelka et al., 2005). During embryogenesis, MECP2 is important for neuronal cell fate refinement, cortical development, and neuronal maturation (Bedogni et al., 2016; Cobolli Gigli et al., 2018; Stefanelli et al., 2016). When MECP2 is missing during embryogenesis, there is a delay in neuronal maturation due to the dysregulated expression of genes that determine the identity of proliferating neural progenitors (Cobolli Gigli et al., 2018). MECP2 is also important for continued neuronal maintenance and function throughout postnatal development and adulthood (McGraw et al., 2011; Nguyen et al., 2012). Researchers removed MECP2 at different age points from a conditional *Mecp2* knockout mouse and identified multiple different time points in which the structure and function of neurons was particularly sensitive to MECP2 levels in the brain, with both groups finding a sensitive period between 8-14 weeks of age (Cheval et al., 2012; Du et al., 2016).

MECP2 deficiency causes morphological changes in dendritic morphology and spine formation in the cortex (Armstrong et al., 1995; Belichenko et al., 2009; Rietveld et al., 2015). In 1995, a study of RTT patient tissue showed that dendrites in certain cortical regions were shorter than in non-RTT brains (Armstrong et al., 1995). Follow up studies in the developing *Mecp2*-null male showed a reduction in dendritic length and dendritic spine density in the motor and barrel field cortices (Belichenko et al., 2009; Lee et al., 2017). These studies suggest that neuron morphological changes may be causing functional issues later in development, contributing to the symptoms

seen in the male model. In the *Mecp2*-Het, morphological changes occur in a cell-autonomous fashion. For example, morphological changes such as reduction in dendritic length, branching and soma area, were seen in the MECP2- (negative) neurons, similar to that of the *Mecp2*-null, but not in the MECP2+ (positive) neurons, within the motor cortex of adult animals (~ 6 months old). However, there were also non-cell autonomous changes that were occurring in the MECP2+ Het neurons, like overall reduction in soma size when compared to WT controls (Belichenko et al., 2009; Rietveld et al., 2015). Together, these data suggest that the later onset of behavioral phenotypes in the Het may be due to the compensatory function of the MECP2+ neurons, which gradually change with age due to interactions with the MECP2- neurons. To investigate this further, developmental time points will need to be added to see if MECP2+ neuron morphology changes over time, as these studies were done in mid-late adulthood.

Functionally, loss of MECP2 from neuronal tissue shifts the excitation/inhibition balance in favor of inhibition (Chao et al., 2010; Dani et al., 2005; Durand et al., 2012; Moretti, 2006). MECP2, which is an activity-dependent gene expression regulator, directly occupies the promoter regions of the genes that make GAD67 (the enzyme that synthesizes GABA) and *parvalbumin* (*PV*), a calcium binding protein that is a marker of a sub-type of inhibitory neuron (Chao et al., 2010; Durand et al., 2012). When *Mecp2* is removed from GABAergic neurons, it recapitulates behavioral phenotypes seen in the *Mecp2*-null mouse model (Chao et al., 2010). When *Mecp2* is removed specifically from PV+ GABAergic interneurons, these animals did not exhibit all of the syndromic phenotypes of the *Mecp2*-null; however, experience-dependent plasticity within the cortex during developmentally important periods, like the visual critical period, was abolished (He et al., 2014). The visual critical period is a specific period of heightened plasticity in the visual cortex that is initiated by eye-opening (visual experience) in which binocular matching occurs. This process is reliant on the timely maturation of the GABAergic circuit, as are many cortical experience-dependent plasticity events (Fagiolini and Hensch, 2000; Hensch, 2004). Based on these findings, one hypothesis of RTT pathogenesis is that MECP2 regulates experience-dependent

plasticity in specific neural circuits during windows of enhanced sensory experience throughout life. MECP2 deficiency causes disruptions in timing of these plasticity mechanisms, resulting in deficits in behavioral output.

During development in the visual critical period, normal maturation of the circuit is characterized by a gradual increase in expression of PV and perineuronal net structures, that inhibit synaptic plasticity, after vision onset at 15 days old (P15) correlating with an increase in inhibitory transmission which reaches a peak at P30 (Hensch, 2005). In the *Mecp2*-null male mouse, eye opening caused the precocious increase in expression of inhibitory markers, such as GAD67, PV and perineuronal nets at P15, which was still higher than WT at P30. This indicated the early closure of the critical period and resulted in mismatched binocular vision (Krishnan et al., 2015). A similar dysregulation of PV-mediated experience-dependent plasticity during sensorimotor integration as seen in the *Mecp2*-null motor cortex (Morello et al., 2018). Precocious maturation of the GABAergic network, in the insular cortex, involved in tactile-auditory sensory integration, was noted by reduced activity in the region during multisensory stimulation (Gogolla et al., 2014).

In an adult learning paradigm, termed the pup retrieval assay, experience-dependent plasticity was restricted in the adult *Mecp2*-Het auditory cortex which caused the Het to inefficiently perform the behavior (Krishnan et al., 2017). Just as in the developing visual cortex of the *Mecp2*-null, the inhibitory network was dysregulated as described by the increased expression of PV as well as the increased expression of perineuronal nets after the behavior (Krishnan et al., 2017). Together, these studies suggest that the dysregulation of experience-dependent plasticity, as marked by increased expression of PV, GAD67, and perineuronal nets, is a common motif in RTT pathogenesis.

Perineuronal Nets

Perineuronal nets (PNNs) are extracellular matrix structures that surround the soma and proximal dendrites of (predominantly) PV+ neurons after a new experience (Hartig et al., 1992). PNNs are a marker of restricted plasticity in both development

and adulthood, as they form after an experience-dependent event solidifying the new neural connections that have been made (Hou et al., 2017; Miyata and Kitagawa, 2017; Pizzorusso et al., 2002). The formation of these structures is often dysregulated in neurological disorders, generally surrounding the PV+ neuron too early and preventing new neural connections from forming (Banerjee et al., 2017; Berretta et al., 2015; Krishnan et al., 2015, 2017). These structures have many functions, and multiple different ways of regulating plasticity, in the brain; most of which are linked to their composition.

PNNs are made from over 40 different extracellular proteins and molecules that fall into 4 main categories: chondroitin sulfate proteoglycans (CSPGs), link proteins, tenascin-r, and hyaluronan (Miyata and Kitagawa, 2017). Hyaluronan is a glycosaminoglycan that acts as a backbone for the aggregation of the other PNN components around the neuron (Bignami et al., 1992; Miyata and Kitagawa, 2017; Sorg et al., 2016; Ueno et al., 2018a). Link proteins, like hyaluronan and proteoglycan binding protein 1, help facilitate interactions between hyaluronan and the CSPGs (Oohashi et al., 2015). Tenascin-R connects CSPGs together within the structure helping to form the reticular-like structure that is indicative of a mature PNN (Brückner et al., 2000; Pesheva and Probstmeier, 2000). Most of the PNN structure is composed of varying CSPGs, which are composed of sulfonated glycosaminoglycans that are attached to a protein core. Wisteria floribunda agglutinin (WFA), commonly used as a marker for PNNs, specifically binds to N-acetyl galactosamine found on most chondroitin sulfate side chains of CSPGs (Brückner et al., 1996; Hartig et al., 1992). WFA specifically binds to N-acetyl galactosamine found on most chondroitin sulfate side chains of CSPGs. Not every PNN contains the same CSPG make up and this heterogeneity has led to many different proposed functions for these structures in the brain (Ueno et al., 2018a, 2018a). These CSPGs have been shown to contribute to the function of the PNN, whether it is a shift in the sulfonation levels of the glycosaminoglycan side chains or localization to a specific synapse for stabilization (Beurdeley et al., 2012; Dick et al., 2013; Favuzzi et al., 2017).

For example, brevican, one of the many CSPGs, is present in PNNs that form around PV+ interneurons in the visual cortex. Specifically, brevican localizes to areas on the PV+ cell that are receiving excitatory input, modulating the location of AMPA receptors within the PV+ neurons membrane (Favuzzi et al., 2017). Additionally, the physical barrier of the net-like structure can act as an ionic and physical buffer for toxic proteins and ions (harmful or not) that are trying to enter the cell (Sorg et al., 2016; Suttikus et al., 2017). CSPGs can bind to chemorepulsive extracellular matrix molecules, like Semaphorin3A, which prevent growth cones from getting close to the PV+ neuron's membrane, which aids in the PNN's physical barrier function (Dick et al., 2013; Vo et al., 2013). The CSPGs can also bind to molecules, like transcription factor Otx2, bringing them closer to the cell membrane (Bernard and Prochiantz, 2016; Sugiyama et al., 2008).

PNNs can be found in nearly every region of the brain, at one time point or another (Ueno et al., 2018b). Their functionality was initially characterized in the visual cortex during the visual critical period in development. Mature high-intensity PNNs are thought to be inhibitory to experience-dependent plasticity, as their increase in developing primary visual cortex correlates with the termination of the critical period and binocular matching. When mice are monocularly deprived (closing of one eye during the critical period), the neurons in the binocular zone of the visual cortex respond only to the eye that was open during the critical period and not the one that was deprived (Fagiolini and Hensch, 2000). Since that circuit has matured based on the sensory input from the one eye, visual input from the deprived eye after the termination of the critical period is not processed in this region. However, when PNNs in this region are enzymatically degraded by the injection of chondroitinase ABC into the adult mature visual cortex, plasticity is restored to the region and binocular matching is established (Pizzorusso et al., 2002, 2006). This study not only identified PNNs as markers for inhibited plasticity but as a substrate for controlling structural plasticity of a whole brain region and circuit (Pizzorusso et al., 2002).

Additional studies in the developing and adult cortex, hippocampus and amygdala have shown that PNNs are dysregulated in varying neurological disorders.

In these contexts, enzymatic degradation of PNNs re-established plasticity to the region and rescued the behavioral phenotype being measured (Berretta et al., 2015; Krishnan, Lau et al., 2017; Pizzo et al., 2016; Reinhard et al., 2019). In the RTT mouse model, PNN expression is increased in the visual cortex during the visual critical period, resulting in mismatched binocular vision (Krishnan et al., 2015). PNN expression was also increased in the Het auditory cortex after the learned maternal behavior, resulting in inefficient pup retrieval (Krishnan et al., 2017). When PNNs were enzymatically degraded from the auditory cortex, the Het's behavior improved in comparison to before the injection but did not significantly improve in comparison to WT (Krishnan et al., 2017). The pup retrieval behavior is a complex, multisensory paradigm that involves the integration of auditory, olfactory, and tactile sensory cues (Stern, 1990). The partial rescue of the pup retrieval phenotype suggested that another brain region involved in the execution of this behavior may be undergoing a similar dysregulation of experience-dependent plasticity in the SurHet.

The Pup Retrieval Paradigm

Maternal behavior

The term 'maternal behaviors' encompasses different etiologically-relevant social behaviors involving but not limited to nursing, pup grooming and pup retrieval. These stereotypic pup-directed behaviors are aimed at the nourishment, care, and safety of the pups (Beach and Jaynes, 1954). The onset of maternal behaviors in first-time dams is dependent on both hormonal cues caused by pregnancy and non-hormonal cues from pup interaction (Stolzenberg and Champagne, 2016). The underlying circuitry of maternal behaviors has been well characterized over the years, though the bulk of literature has focused on sub-cortical sensory integration regions like the medial preoptic area, medial and basolateral amygdala, ventral tegmental area, and the periaqueductal gray (reviewed in Kohl et al., 2017; Lonstein et al., 2015). These sub-cortical regions are involved in the integration of olfactory, auditory, and tactile sensory cues from the pups, which stimulate certain behaviors from the adult (Stern, 1990).

Pup Retrieval

Pup retrieval involves the processing of pup ultrasonic vocalizations and odor to properly locate the pups outside of the nest (Banerjee and Liu, 2013; Cohen et al., 2011a; Okabe et al., 2013). Tactile feedback from the whiskers is required for navigating the enclosure to find the pup, then tactile input from the perioral region (whiskers, upper lip, and jaw) are required for identifying and picking up the pup to bring back to the nest (Kenyon et al., 1981; Morgan et al., 1992). This paradigm also has a learning component, as the adults have to learn what the USV distress calls mean and that the pups need to be retrieved back to the nest (Banerjee and Liu, 2013; Cohen and Mizrahi, 2015). First-time dams efficiently perform this behavior after the pups are born, as it is a major component of maternal care. Virgin female mice with no previous pup exposure or maternal experiences (Naïve) learn and efficiently perform this behavior after being co-housed (then termed surrogates) with a first-time dam and her pups (Alsina-Llanes et al., 2015; Cohen et al., 2011a). Cohen et al., found that the integration pup odor and ultrasonic vocalizations induced experience-dependent plasticity changes within the auditory cortex of the Surrogate (Sur) females (Cohen et al., 2011a).

The pup retrieval assay is an etiologically relevant social behavior that induces experience-dependent plasticity changes in the adult mouse brain, independent of hormonal regulation (Stolzenberg and Champagne, 2016; Stolzenberg and Rissman, 2011). When this behavior was done with adult *Mecp2*-Het female mice, the SurHets spent significantly more time retrieving the pups back to the nest and made significantly more errors, pup interactions that failed to end in retrieval, in bringing the pups back to the nest (Krishnan et al., 2017). This was caused by a dysregulation of the inhibitory circuit in the SurHet which restricted experience-dependent plasticity in the auditory cortex (Krishnan et al., 2017; Lau et al., 2020). When plasticity was restored to the auditory cortex, via the enzymatic degradation of PNNs by chABC injection, the SurHets were able to perform more efficiently at the pup retrieval paradigm than those without the chABC injection (Krishnan et al., 2017). However, they still were not as efficient as the SurWT controls suggesting that experience-dependent plasticity in another region or sensory circuit is being dysregulated in the SurHet.

Multisensory paradigm

Olfaction

In addition to auditory cues, olfactory cues, and tactile sensory input from the pups, whether it be from nursing, grooming or during pup retrieval is important for the onset of maternal behavior, in first-time dams as well as surrogate female mice (Beach and Jaynes, 1954; Stern, 1990; Walsh et al., 1996). Pup odor is important for the onset of maternal behaviors in first-time dams; however, the olfactory sensory circuit is complex. For example, manipulations to the main olfactory epithelium result in pup retrieval deficits (longer to gather pups to the nest), while manipulations to the vomeronasal organ had no effect on maternal behavior onset (Fraser and Shah, 2014; Weiss et al., 2011). After physical exposure to pups, surrogate females showed increased expression levels of c-Fos (a marker of neuronal activity) in the olfactory nucleus, piriform cortex, medial/basolateral/central amygdala, and entorhinal cortex (Calamandrei and Keverne, 1994; Okabe et al., 2013). Olfactory desensitization decreases Fos expression in the olfactory bulb, piriform cortex, and olfactory tubercle (Walsh et al., 1996). These changes in the olfactory circuit, along with the important combination of pup odor with pup calls in the learning of the pup gathering behavior (Cohen et al., 2011a), identify the olfactory circuit as a potential circuit that could have dysregulated experience-dependent plasticity in the adult SurHet.

Somatosensation

Somatosensation through tactile feedback from pups is particularly important for the development of maternal behavior; pup gathering in particular (Stern, 1990). Tactile feedback from the pups through the perioral area (snout and whiskers) and the ventral trunk are required for the onset of pup retrieval and other pup-directed behaviors. When tactile input from the perioral area or the ventral trunk is blocked, through lidocaine injections and ventral wraps, pup retrieval efficiency is disrupted and the rodents take longer to learn how to efficiently retrieve the pups (Kenyon et al., 1981; Morgan et al., 1992; Stern and Kolunje, 1989). However, when somatosensory input from both the perioral area and ventral trunk is restricted, the pup retrieval behavior is nearly abolished (Morgan et al., 1992). Tactile feedback from pups is also essential for the onset of maternal behaviors in previously Naïve female mice (Alsina-Llanes et al.,

2015). Tactile input from the pups induces increased c-Fos expression in the periaqueductal gray, which when lesioned disrupts pup retrieval behaviors (Lonstein and Stern, 1997), as well as in the somatosensory cortex, which has not been well studied in the context of maternal behavior (Lonstein et al., 1997).

The somatosensory cortex processes tactile input from all over the body (Feldman and Brecht, 2005). It also integrates sensory information from other cortical regions in a sensorimotor capacity (Kinnischtzke et al., 2014; Lee et al., 2008; Mathis et al., 2017; Sreenivasan et al., 2015) and during multisensory social behaviors (Bobrov et al., 2014; Keniston et al., 2010; Rao et al., 2014). In male *Mecp2*-null mice, neurons in the somatosensory cortex have atypical morphology and an overall smaller representative area than age-matched WTs (as described in 'MECP2 in neurons') (Lee et al., 2017). Additionally, atypical plasticity changes, like increased expression of PV, were seen in the null somatosensory cortex during a sensorimotor experience-dependent event (Morello et al., 2018). While both of these studies were done in *Mecp2*-null males, both show that the somatosensory cortex is affected by MECP2 deficiency, highlighting the somatosensory cortex as a region of interest through which to study adult experience-dependent plasticity mechanisms in both the social pup retrieval paradigm and in basic tactile perception in the Het.

Primary Somatosensory Cortex and Tactile Sensation

Somatosensory input is processed in the primary somatosensory cortex (SS1), a large region of the rodent cortex that receives tactile information the body and integrates sensory information from other cortical brain regions (Feldman and Brecht, 2005; Welker et al., 1992; Xerri et al., 1994, 2005). Tactile information from the periphery travels to layer IV of the SS1 via thalamocortical input (Woolsey and Van der Loos, 1970). This information is then sent to layer V & VI projection neurons for sensory information output to subcortical regions (Petersen, 2007), or to layer II & III neurons which facilitate long-range cortico-cortical connections between the SS1 and other sensory regions (Aronoff et al., 2010; Chen et al., 2013; Kinnischtzke et al., 2014; Rao et al., 2014). The SS1 is split into functional subregions that receive tactile input from

specific regions of the body, such as the S1FL (forelimb) which processes tactile input from the forepaws (Xerri et al., 2005), S1Tr (trunk) which receives input from the trunk of the mouse (Xerri et al., 1994), S1DZ (dysgranular zone) which has been implicated in sensorimotor integration (Chapin and Lin, 1984; Lee and Kim, 2012; Welker et al., 1984), and S1BF (barrel field cortex) which receives tactile input from this whiskers (Axelrad et al., 1976; Durham and Woolsey, 1978). The majority of the research on the SS1 has been done within the S1BF, due to the importance of whiskers in navigating the rodent environment (Arkley et al., 2017), social interactions (Bobrov et al., 2014; Bolognini et al., 2014) and its distinct cytoarchitecture (Woolsey and Van der Loos, 1970).

Plasticity in the Primary Somatosensory Cortex

The S1BF was named the barrel field cortex based on the barreloid structures that form in subregion. Each barrel receives thalamocortical input from the ventral posterior medial nucleus of the thalamus (VPM) into layer IV of the cortex from an individual whisker, this connectivity is outlined in Figure 3 (Durham and Woolsey, 1978; Welker et al., 1992; Woolsey and Van der Loos, 1970). The S1BF is often used as a model of activity-dependent plasticity, like the developing visual cortex, but plasticity in the S1BF can be studied at any time point. When whiskers are removed from the mystacial pad (the muscle that the whiskers are attached to) the barrel that corresponds to that whisker is now deprived of sensory input – which causes the representation of that barrel to shrink. If the neighboring barrels are still receiving thalamocortical input, their representation expands to use those cortical neurons that are not receiving VPM input. As the removed whisker grows back, those thalamocortical projections begin to send information to the deprived barrel, and the representation of that barrel comes back (Kossut, 1998; Maier et al., 2003; Wallace and Fox, 1999). As activity via thalamocortical input, or a lack thereof, causes cortical experience-dependent plasticity changes in barrel formation and representation, other researchers explored what plasticity changes would happen within the S1BF if specific whiskers were constantly stimulated.

Early studies of constant whisker stimulation found that there was an increase in GAD-positive neurons on the periphery of the individual barrels after 4 days of constant stimulation (Welker et al., 1989). As this study was over a long period of time, a similar experiment was done with 24 hours of constant stimulation of a subset of barrels within the cortex. Directly after that 24-hour period, synaptic density was significantly increased in the barrels that had been stimulated, but not in the control barrels of the same animal. Specifically, there was an increase in spine density with only excitatory synapses as well as those with excitatory and GABAergic synapses. Four days post-stimulation, the spine density and number of excitatory synapses returned to basal level (pre-stimulation) while the increased GABAergic synapses, particularly in layer IV, were maintained (Knott et al., 2002).

Another connection to inhibitory control of plasticity in the SS1 are multiple studies that have characterized the expression of PNNs and PV during the development of the S1BF and how sensory deprivation effects the expression of these inhibitory markers. The number of PV+ interneurons and PNN+ neurons in the S1BF significantly increases over the first 20 days of life, with a more significant number of those being within layer IV (Nowicka et al., 2009; Ueno et al., 2017). Sensory deprivation, by full whisker trimming from birth, had no significant change in PV+ neurons, however there were significantly less PV+/PNN+ neurons by post-natal day 56, which is generally considered adulthood, when compared to the control animals that had no sensory deprivation. Additionally, the PNNs in the deprived S1BF were not mature PNNs as they had a more diffuse structure typical of PNNs that are in the process of forming, rather than the reticular structure of mature PNNs (Ueno et al., 2017). Together, these studies suggest that experience-dependent plasticity in the SS1 may be mediated by the inhibitory network, similar to the visual and auditory cortices. In addition to sensory deprivation and sensory enrichment, the SS1 has also been studied in the context of learning, sensory navigation, and social behaviors.

Whisker-based tactile sensation

Tactile sensory information from the whiskers is important for the survival of the animal as it is one of the primary senses that rodents use to navigate their environment

(Anjum and Brecht, 2012; Arkley et al., 2017; Brecht, 2007; Diamond et al., 2008). For example, the dormouse is one of a few rodent species that travels across the tops of trees in order to avoid open spaces where it can be easily hunted. In this context, the dormouse uses its whiskers to sense the gap between them and the next branch, sensing both the end of the current branch as well as judging the distance needed to jump to the next branch, while protracting their whiskers during flight to aid in a safe and balanced landing (Arkley et al., 2017). Whiskers can also be used in prey-capture behaviors, though the role of whisking in this context has been best studied in the shrew, a nocturnal rodent that relies heavily on its whiskers to sense its environment (Anjum and Brecht, 2012; Anjum et al., 2006; Munz et al., 2010). The tactics the shrew uses to hunt its prey are disrupted, along with reduced success, when the whiskers are trimmed at an early age (Anjum and Brecht, 2012).

In a lab setting, the function of whiskers and the dynamics of how whisking can help rodents engage with their environment are studied through more simple texture discrimination tasks. Texture discrimination was first shown to be a simple whisker-mediated behavior when whiskers were removed from rodents who had been trained to identify a specific texture. After removal they were unable to differentiate between the texture they had been trained to recognize and the other texture in the paradigm (Guic-Robles et al., 1989). The bilateral lesioning of the barrel cortex produced a similar result (Guic-Robles et al., 1992). Most studies that focus on the neural basis of whisker-dependent changes in the S1BF are done using head-fixed animals for electrophysiological or intrinsic imaging experiments (He et al., 2017; Lee et al., 2017; Masino et al., 1993). These animals are trained to recognize a specific texture, supplied by the researcher, through go-no go tasks. They are then tested with multiple different textures and, if they can differentiate between them, will indicate when they sense the correct texture (Kuhlman et al., 2014; Lee et al., 2019; O'Connor et al., 2010; Park et al., 2020). This has even been done with virtual reality, to make the behavior more etiologically relevant to the animal while maintaining the head-fixed methodology (Sofroniew et al., 2014).

As behavioral studies move more toward etiologically relevant behavioral assays, there has been a renewed interest in studying free moving, untrained, animals in these texture discrimination tasks. Adapted from the novel object recognition task (Ennaceur and Delacour, 1988) this free moving assay tests texture discrimination by measuring the time that the subject spends interacting with a 'novel' texture versus a texture that they had been habituated to (Pacchiarini et al., 2017; Wu et al., 2013). While mostly used to test whisker-based tactile sensory processing, the texture discrimination task has also been used to test peripheral tactile sensory deficits via tactile investigation through the forepaw. This study, done on whiskerless symptomatic *Mecp2*-null male mice, showed that the males were unable to perform the behavior, which is to differentiate between the novel and familiar textures, based on peripheral input (Orefice et al., 2016). Whisker-based deficits were not explored in this paper, despite there being known structural and functional deficits in the null barrel cortex (Lee et al., 2017; Moroto et al., 2013).

Somatosensation and Social Behaviors

Tactile somatosensory input is important during social interactions, as was previously outlined in the pup retrieval behavior (*parental behavior section*). During social behavior, both whisker interactions and facial touch are represented within the SS1. These studies are done in a gap test – where the rodents are separated by a wide enough gap that they can only interact with their whiskers and noses (alternative set ups have the researcher holding the stimulus rodent) (Bobrov et al., 2014; Lenschow and Brecht, 2015; Rao et al., 2014). Whisker interactions during the social paradigm induced a larger change in membrane potential in the SS1 than whisker interactions with a stationary object (Bobrov et al., 2014; Lenschow and Brecht, 2015). Social touch was also associated with increased auditory communication (through USVs) between the two rodents. Tactile input from the whiskers modulated activity in the auditory cortex (Rao et al., 2014), which matches the large changes in membrane potential of SS1 during social whisking, seen in layers II & III, which are involved in sensory cortico-cortical connectivity (Lenschow and Brecht, 2015).

Together these results suggest a functional somatosensory-auditory sensory integration pathway exists during social behaviors, similar to the olfactory-auditory integration seen during maternal behavior onset. Based on what is known about the somatosensory cortex's GABA-mediated activity-dependent plasticity as well as the structural and functional deficits seen in the *Mecp2*-null male; the SS1 is a great region in which to study the effect of MECP2 deficiency (Het) on adult experience-dependent plasticity. Due to its role in tactile sensation, multisensory integration, the importance of somatosensation to the pup retrieval behavior, the effect of MECP2 deficiency on experience-dependent plasticity within both social and asocial contexts can be explored. The regulation of pup retrieval behavior through SS1 provides an etiologically relevant approach for testing the hypothesis generated from the developing visual (He et al., 2014; Krishnan et al., 2015) and motor (Morello et al., 2018) cortices as well as the adult auditory cortex (Krishnan et al., 2017), that MECP2 regulates the timing of experience-dependent plasticity mechanisms during periods of enhanced sensory and social experience throughout life.

References

- Alsina-Llanes, M., Victoria De Brun, and Olazábal, D.E. (2015). Development and expression of maternal behavior in naïve female C57BL/6 mice: Ontogeny of Maternal Behavior in Mice. *Developmental Psychobiology* 57, 189–200.
- Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* 23, 185–188.
- Anjum, F., and Brecht, M. (2012). Tactile experience shapes prey-capture behavior in Etruscan shrews. *Frontiers in Behavioral Neuroscience* 6.
- Anjum, F., Turni, H., Mulder, P.G.H., van der Burg, J., and Brecht, M. (2006). Tactile guidance of prey capture in Etruscan shrews. *Proceedings of the National Academy of Sciences* 103, 16544–16549.
- Arkley, K., Tiktak, G.P., Breakell, V., Prescott, T.J., and Grant, R.A. (2017). Whisker touch guides canopy exploration in a nocturnal, arboreal rodent, the Hazel dormouse (*Muscardinus avellanarius*). *Journal of Comparative Physiology A* 203, 133–142.
- Armstrong, D., Dunn, J.K., Antalffy, B., and Trivedi, R. (1995). Selective Dendritic Alterations in the Cortex of Rett Syndrome: *Journal of Neuropathology and Experimental Neurology* 54, 195–201.

Aronoff, R., Matyas, F., Mateo, C., Ciron, C., Schneider, B., and Petersen, C.C.H. (2010). Long-range connectivity of mouse primary somatosensory barrel cortex: Long-range connectivity of barrel cortex. *European Journal of Neuroscience* 31, 2221–2233.

Axelrad, H., Verley, R., and Farkas, E. (1976). Responses evoked in mouse and rat SI cortex by vibrissa stimulation. *Neuroscience Letters* 3, 265–274.

Banerjee, S.B., and Liu, R.C. (2013). Storing maternal memories: Hypothesizing an interaction of experience and estrogen on sensory cortical plasticity to learn infant cues. *Frontiers in Neuroendocrinology* 34, 300–314.

Banerjee, S.B., Gutzeit, V.A., Baman, J., Aoued, H.S., Doshi, N.K., Liu, R.C., and Ressler, K.J. (2017). Perineuronal Nets in the Adult Sensory Cortex Are Necessary for Fear Learning. *Neuron* 95, 169-179.e3.

Beach, F.A., and Jaynes, J. (1954). Effects of early experience upon the behavior of animals. *Psychol Bull* 51, 239–263.

Bedogni, F., Cobolli Gigli, C., Pozzi, D., Rossi, R.L., Scaramuzza, L., Rossetti, G., Pagani, M., Kilstrup-Nielsen, C., Matteoli, M., and Landsberger, N. (2016). Defects During *Mecp2* Null Embryonic Cortex Development Precede the Onset of Overt Neurological Symptoms. *Cereb. Cortex* 26, 2517–2529.

Belichenko, N.P., Belichenko, P.V., and Mobley, W.C. (2009). Evidence for both neuronal cell autonomous and nonautonomous effects of methyl-CpG-binding protein 2 in the cerebral cortex of female mice with *Mecp2* mutation. *Neurobiology of Disease* 34, 71–77.

Bernard, C., and Prochiantz, A. (2016). Otx2-PNN Interaction to Regulate Cortical Plasticity. *Neural Plasticity* 2016, 1–7.

Berretta, S., Pantazopoulos, H., Markota, M., Brown, C., and Batzianouli, E.T. (2015). Losing the sugar coating: Potential impact of perineuronal net abnormalities on interneurons in schizophrenia. *Schizophrenia Research* 167, 18–27.

Beurdeley, M., Spatazza, J., Lee, H.H.C., Sugiyama, S., Bernard, C., Di Nardo, A.A., Hensch, T.K., and Prochiantz, A. (2012). Otx2 Binding to Perineuronal Nets Persistently Regulates Plasticity in the Mature Visual Cortex. *Journal of Neuroscience* 32, 9429–9437.

Bignami, A., Asher, R., and Perides, G. (1992). Co-localization of hyaluronic acid and chondroitin sulfate proteoglycan in rat cerebral cortex. *Brain Res.* 579, 173–177.

Bobrov, E., Wolfe, J., Rao, R.P., and Brecht, M. (2014). The Representation of Social Facial Touch in Rat Barrel Cortex. *Current Biology* 24, 109–115.

Bolognini, N., Rossetti, A., Fusaro, M., Vallar, G., and Miniussi, C. (2014). Sharing Social Touch in the Primary Somatosensory Cortex. *Current Biology* 24, 1513–1517.

Brecht, M. (2007). Barrel cortex and whisker-mediated behaviors. *Curr. Opin. Neurobiol.* 17, 408–416.

- Brown, K., Selfridge, J., Lagger, S., Connelly, J., De Sousa, D., Kerr, A., Webb, S., Guy, J., Merusi, C., Koerner, M.V., et al. (2016). The molecular basis of variable phenotypic severity among common missense mutations causing Rett syndrome. *Hum. Mol. Genet.* 25, 558–570.
- Brückner, G., Bringmann, A., Köppe, G., Härtig, W., and Brauer, K. (1996). In vivo and in vitro labelling of perineuronal nets in rat brain. *Brain Res.* 720, 84–92.
- Brückner, G., Grosche, J., Schmidt, S., Härtig, W., Margolis, R.U., Delpech, B., Seidenbecher, C.I., Czaniera, R., and Schachner, M. (2000). Postnatal development of perineuronal nets in wild-type mice and in a mutant deficient in tenascin-R. *J. Comp. Neurol.* 428, 616–629.
- Buchanan, C.B., Stallworth, J.L., Scott, A.E., Glaze, D.G., Lane, J.B., Skinner, S.A., Tierney, A.E., Percy, A.K., Neul, J.L., and Kaufmann, W.E. (2019). Behavioral profiles in Rett syndrome: Data from the natural history study. *Brain and Development* 41, 123–134.
- Calamandrei, G., and Keverne, E.B. (1994). Differential expression of Fos protein in the brain of female mice dependent on pup sensory cues and maternal experience. *Behavioral Neuroscience* 108, 113–120.
- Chahrour, M., and Zoghbi, H.Y. (2007). The Story of Rett Syndrome: From Clinic to Neurobiology. *Neuron* 56, 422–437.
- Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T.C., Qin, J., and Zoghbi, H.Y. (2008). MeCP2, a Key Contributor to Neurological Disease, Activates and Represses Transcription. *Science* 320, 1224–1229.
- Chao, H.-T., Chen, H., Samaco, R.C., Xue, M., Chahrour, M., Yoo, J., Neul, J.L., Gong, S., Lu, H.-C., Heintz, N., et al. (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* 468, 263–269.
- Chapin, J.K., and Lin, C.S. (1984). Mapping the body representation in the SI cortex of anesthetized and awake rats. *J. Comp. Neurol.* 229, 199–213.
- Chen, J.L., Carta, S., Soldado-Magraner, J., Schneider, B.L., and Helmchen, F. (2013). Behaviour-dependent recruitment of long-range projection neurons in somatosensory cortex. *Nature* 499, 336–340.
- Cheval, H., Guy, J., Merusi, C., De Sousa, D., Selfridge, J., and Bird, A. (2012). Postnatal inactivation reveals enhanced requirement for MeCP2 at distinct age windows. *Human Molecular Genetics* 21, 3806–3814.
- Cobolli Gigli, C., Scaramuzza, L., De Simone, M., Rossi, R.L., Pozzi, D., Pagani, M., Landsberger, N., and Bedogni, F. (2018). Lack of Methyl-CpG Binding Protein 2 (MeCP2) Affects Cell Fate Refinement During Embryonic Cortical Development. *Cerebral Cortex* 28, 1846–1856.
- Cohen, L., and Mizrahi, A. (2015). Plasticity during Motherhood: Changes in Excitatory and Inhibitory Layer 2/3 Neurons in Auditory Cortex. *Journal of Neuroscience* 35, 1806–1815.

- Cohen, L., Rothschild, G., and Mizrahi, A. (2011a). Multisensory integration of natural odors and sounds in the auditory cortex. *Neuron* 72, 357–369.
- Cohen, S., Gabel, H.W., Hemberg, M., Hutchinson, A.N., Sadacca, L.A., Ebert, D.H., Harmin, D.A., Greenberg, R.S., Verdine, V.K., Zhou, Z., et al. (2011b). Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. *Neuron* 72, 72–85.
- Dani, V.S., Chang, Q., Maffei, A., Turrigiano, G.G., Jaenisch, R., and Nelson, S.B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 102, 12560–12565.
- Dayer, A.G., Bottani, A., Bouchardy, I., Fluss, J., Antonarakis, S.E., Haenggeli, C.-A., and Morris, M.A. (2007). MECP2 mutant allele in a boy with Rett syndrome and his unaffected heterozygous mother. *Brain and Development* 29, 47–50.
- Diamond, M.E., von Heimendahl, M., and Arabzadeh, E. (2008). Whisker-Mediated Texture Discrimination. *PLoS Biology* 6, e220.
- Dick, G., Tan, C.L., Alves, J.N., Ehlert, E.M.E., Miller, G.M., Hsieh-Wilson, L.C., Sugahara, K., Oosterhof, A., van Kuppevelt, T.H., Verhaagen, J., et al. (2013). Semaphorin 3A Binds to the Perineuronal Nets via Chondroitin Sulfate Type E Motifs in Rodent Brains. *Journal of Biological Chemistry* 288, 27384–27395.
- Djukic, A., and Valicenti McDermott, M. (2012). Social Preferences in Rett Syndrome. *Pediatric Neurology* 46, 240–242.
- Du, F., Nguyen, M.V.C., Karten, A., Felice, C.A., Mandel, G., and Ballas, N. (2016). Acute and crucial requirement for MeCP2 function upon transition from early to late adult stages of brain maturation. *Hum. Mol. Genet.* 25, 1690–1702.
- Durand, S., Patrizi, A., Quast, K.B., Hachigian, L., Pavlyuk, R., Saxena, A., Carninci, P., Hensch, T.K., and Fagiolini, M. (2012). NMDA receptor regulation prevents regression of visual cortical function in the absence of Mecp2. *Neuron* 76, 1078–1090.
- Durham, D., and Woolsey, T.A. (1978). Acute whisker removal reduces neuronal activity in barrels of mouse smI cortex. *J. Comp. Neurol.* 178, 629–644.
- Ebert, D.H., Gabel, H.W., Robinson, N.D., Kastan, N.R., Hu, L.S., Cohen, S., Navarro, A.J., Lyst, M.J., Ekiert, R., Bird, A.P., et al. (2013). Activity-Dependent Phosphorylation of MeCP2 T308 Regulates Interaction with NCoR. *Nature* 499, 341–345.
- Ennaceur, A., and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research* 31, 47–59.
- Fagiolini, M., and Hensch, T.K. (2000). Inhibitory threshold for critical-period activation in primary visual cortex. *Nature* 404, 183–186.
- Favuzzi, E., Marques-Smith, A., Deogracias, R., Winterflood, C.M., Sánchez-Aguilera, A., Mantoan, L., Maeso, P., Fernandes, C., Ewers, H., and Rico, B. (2017). Activity-Dependent Gating of Parvalbumin Interneuron Function by the Perineuronal Net Protein Brevican. *Neuron* 95, 639–655.e10.

- Feldman, D.E., and Brecht, M. (2005). Map plasticity in somatosensory cortex. *Science* 310, 810–815.
- FitzGerald, P.M., Jankovic, J., and Percy, A.K. (1990). Rett syndrome and associated movement disorders. *Mov Disord.* 5, 195–202.
- Fraser, E.J., and Shah, N.M. (2014). Complex chemosensory control of female reproductive behaviors. *PLoS One* 9, e90368.
- Gogolla, N., Takesian, A.E., Feng, G., Fagiolini, M., and Hensch, T.K. (2014). Sensory Integration in Mouse Insular Cortex Reflects GABA Circuit Maturation. *Neuron* 83, 894–905.
- Guić-Robles, E., Valdivieso, C., and Guajardo, G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. *Behavioural Brain Research* 31, 285–289.
- Guic-Robles, E., Jenkins, W.M., and Bravo, H. (1992). Vibrissal roughness discrimination is barrelcortex-dependent. *Behavioural Brain Research* 48, 145–152.
- Guy, J., Hendrich, B., Holmes, M., Martin, J.E., and Bird, A. (2001). A mouse *Mecp2*-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat. Genet.* 27, 322–326.
- Hagberg, B., Aicardi, J., Dias, K., and Ramos, O. (1983). A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: Report of 35 cases. *Ann Neurol.* 14, 471–479.
- Hartig, W., Brauer, K., and Bruckner, G. (1992). Wisteria floribunda agglutinin-labelled nets surround parvalbumin-containing neurons. *Neuroreport* 3, 869–872.
- He, C.X., Cantu, D.A., Mantri, S.S., Zeiger, W.A., Goel, A., and Portera-Cailliau, C. (2017). Tactile Defensiveness and Impaired Adaptation of Neuronal Activity in the *Fmr1* Knock-Out Mouse Model of Autism. *The Journal of Neuroscience* 37, 6475–6487.
- He, L.J., Liu, N., Cheng, T.L., Chen, X.J., Li, Y.D., Shu, Y.S., Qiu, Z.L., and Zhang, X.H. (2014). Conditional deletion of *Mecp2* in parvalbumin-expressing GABAergic cells results in the absence of critical period plasticity. *Nature Communications* 5, 5036.
- Hensch, T.K. (2004). CRITICAL PERIOD REGULATION. *Annual Review of Neuroscience* 27, 549–579.
- Hensch, T.K. (2005). Critical period mechanisms in developing visual cortex. *Current Topics in Developmental Biology* 69, 215–237.
- Hou, X., Yoshioka, N., Tsukano, H., Sakai, A., Miyata, S., Watanabe, Y., Yanagawa, Y., Sakimura, K., Takeuchi, K., Kitagawa, H., et al. (2017). Chondroitin Sulfate Is Required for Onset and Offset of Critical Period Plasticity in Visual Cortex. *Scientific Reports* 7.
- Humphreys, P., and Barrowman, N. (2016). The Incidence and Evolution of Parkinsonian Rigidity in Rett Syndrome: A Pilot Study. *Can J Neurol Sci* 43, 567–573.

- Jian, L., Nagarajan, L., de Klerk, N., Ravine, D., Bower, C., Anderson, A., Williamson, S., Christodoulou, J., and Leonard, H. (2006). Predictors of seizure onset in Rett syndrome. *The Journal of Pediatrics* 149, 542-547.e3.
- Jung, B.P., Jugloff, D.G.M., Zhang, G., Logan, R., Brown, S., and Eubanks, J.H. (2003). The expression of methyl CpG binding factor MeCP2 correlates with cellular differentiation in the developing rat brain and in cultured cells. *J. Neurobiol.* 55, 86–96.
- Keniston, L.P., Henderson, S.C., and Meredith, M.A. (2010). Neuroanatomical identification of crossmodal auditory inputs to interneurons in somatosensory cortex. *Experimental Brain Research* 202, 725–731.
- Kenyon, P., Cronin, P., and Keeble, S. (1981). Disruption of maternal retrieving by perioral anesthesia. *Physiology & Behavior* 27, 313–321.
- Killian, J.T., Lane, J.B., Lee, H.-S., Skinner, S.A., Kaufmann, W.E., Glaze, D.G., Neul, J.L., and Percy, A.K. (2017). Scoliosis in Rett Syndrome: Progression, Comorbidities, and Predictors. *Pediatric Neurology* 70, 20–25.
- Kinnischtzke, A.K., Simons, D.J., and Fanselow, E.E. (2014). Motor Cortex Broadly Engages Excitatory and Inhibitory Neurons in Somatosensory Barrel Cortex. *Cerebral Cortex* 24, 2237–2248.
- Kishi, N., and Macklis, J.D. (2004). MECP2 is progressively expressed in post-migratory neurons and is involved in neuronal maturation rather than cell fate decisions. *Molecular and Cellular Neuroscience* 27, 306–321.
- Knott, G.W., Quairiaux, C., Genoud, C., and Welker, E. (2002). Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. *Neuron* 34, 265–273.
- Kohl, J., Autry, A.E., and Dulac, C. (2017). The neurobiology of parenting: A neural circuit perspective. *Bioessays* 39, 1–11.
- Kossut, M. (1998). Experience-dependent changes in function and anatomy of adult barrel cortex. *Exp Brain Res* 123, 110–116.
- Krishnan, K., Wang, B.-S., Lu, J., Wang, L., Maffei, A., Cang, J., and Huang, Z.J. (2015). MeCP2 regulates the timing of critical period plasticity that shapes functional connectivity in primary visual cortex. *Proc. Natl. Acad. Sci. U.S.A.* 112, E4782-4791.
- Krishnan, K., Lau, B.Y.B., Ewall, G., Huang, Z.J., and Shea, S.D. (2017). MECP2 regulates cortical plasticity underlying a learned behaviour in adult female mice. *Nature Communications* 8, 14077.
- Kuhlman, S.J., O'Connor, D.H., Fox, K., and Svoboda, K. (2014). Structural Plasticity within the Barrel Cortex during Initial Phases of Whisker-Dependent Learning. *Journal of Neuroscience* 34, 6078–6083.
- Lau, B.Y.B., Krishnan, K., Huang, Z.J., and Shea, S.D. (2020). Maternal Experience-Dependent Cortical Plasticity in Mice Is Circuit- and Stimulus-Specific and Requires MECP2. *The Journal of Neuroscience* 40, 1514–1526.

- Lee, T., and Kim, U. (2012). Descending projections from the dysgranular zone of rat primary somatosensory cortex processing deep somatic input. *J. Comp. Neurol.* 520, 1021–1046.
- Lee, C.R., Yonk, A.J., Wiskerke, J., Paradiso, K.G., Tepper, J.M., and Margolis, D.J. (2019). Opposing Influence of Sensory and Motor Cortical Input on Striatal Circuitry and Choice Behavior. *Current Biology* 29, 1313-1323.e5.
- Lee, L.-J., Tsytsarev, V., and Erzurumlu, R.S. (2017). Structural and functional differences in the barrel cortex of *Mecp2* null mice. *Journal of Comparative Neurology* 525, 3951–3961.
- Lee, S., Carvell, G.E., and Simons, D.J. (2008). Motor modulation of afferent somatosensory circuits. *Nature Neuroscience* 11, 1430–1438.
- Lenschow, C., and Brecht, M. (2015). Barrel Cortex Membrane Potential Dynamics in Social Touch. *Neuron* 85, 718–725.
- Liao, W., Gandal, M.J., Ehrlichman, R.S., Siegel, S.J., and Carlson, G.C. (2012). *MeCP2*^{+/-} mouse model of RTT reproduces auditory phenotypes associated with Rett syndrome and replicate select EEG endophenotypes of autism spectrum disorder. *Neurobiology of Disease* 46, 88–92.
- Lonstein, J.S., and Stern, J.M. (1997). Somatosensory contributions to c-fos activation within the caudal periaqueductal gray of lactating rats: effects of perioral, rooting, and suckling stimuli from pups. *Horm Behav* 32, 155–166.
- Lonstein, J.S., Simmons, D.A., Swann, J.M., and Stern, J.M. (1997). Forebrain expression of c-fos due to active maternal behaviour in lactating rats. *Neuroscience* 82, 267–281.
- Lonstein, J.S., Lévy, F., and Fleming, A.S. (2015). Common and divergent psychobiological mechanisms underlying maternal behaviors in non-human and human mammals. *Hormones and Behavior* 73, 156–185.
- Lyst, M.J., and Bird, A. (2015). Rett syndrome: a complex disorder with simple roots. *Nature Reviews Genetics* 16, 261–275.
- Maier, D.L., Grieb, G.M., Stelzner, D.J., and McCasland, J.S. (2003). Large-scale plasticity in barrel cortex following repeated whisker trimming in young adult hamsters. *Exp Neurol* 184, 737–745.
- Masino, S.A., Kwon, M.C., Dory, Y., and Frostig, R.D. (1993). Characterization of functional organization within rat barrel cortex using intrinsic signal optical imaging through a thinned skull. *Proc. Natl. Acad. Sci. U.S.A.* 90, 9998–10002.
- Masuyama, T., Matsuo, M., Jing, J.J., Tabara, Y., Kitsuki, K., Yamagata, H., Kan, Y., Miki, T., Ishii, K., and Kondo, I. (2005). Classic Rett syndrome in a boy with R133C mutation of MECP2. *Brain and Development* 27, 439–442.
- Mathis, M.W., Mathis, A., and Uchida, N. (2017). Somatosensory Cortex Plays an Essential Role in Forelimb Motor Adaptation in Mice. *Neuron* 93, 1493-1503.e6.

- McGraw, C.M., Samaco, R.C., and Zoghbi, H.Y. (2011). Adult Neural Function Requires MeCP2. *Science* 333, 186–186.
- Metcalf, B.M., Mullaney, B.C., Johnston, M.V., and Blue, M.E. (2006). Temporal shift in methyl-CpG binding protein 2 expression in a mouse model of Rett syndrome. *Neuroscience* 139, 1449–1460.
- Miyata, S., and Kitagawa, H. (2017). Formation and remodeling of the brain extracellular matrix in neural plasticity: Roles of chondroitin sulfate and hyaluronan. *Biochimica et Biophysica Acta* 1861, 2420–2434.
- Morello, N., Schina, R., Pilotto, F., Phillips, M., Melani, R., Plicato, O., Pizzorusso, T., Pozzo-Miller, L., and Giustetto, M. (2018). Loss of Mecp2 Causes Atypical Synaptic and Molecular Plasticity of Parvalbumin-Expressing Interneurons Reflecting Rett Syndrome–Like Sensorimotor Defects. *Eneuro* 5, ENEURO.0086-18.2018.
- Moretti, P. (2006). Learning and Memory and Synaptic Plasticity Are Impaired in a Mouse Model of Rett Syndrome. *Journal of Neuroscience* 26, 319–327.
- Morgan, H.D., Fleming, A.S., and Stern, J.M. (1992). Somatosensory control of the onset and retention of maternal responsiveness in primiparous Sprague-Dawley rats. *Physiology & Behavior* 51, 549–555.
- Moroto, M., Nishimura, A., Morimoto, M., Isoda, K., Morita, T., Yoshida, M., Morioka, S., Tozawa, T., Hasegawa, T., Chiyonobu, T., et al. (2013). Altered somatosensory barrel cortex refinement in the developing brain of Mecp2-null mice. *Brain Research* 1537, 319–326.
- Motil, K.J., Caeg, E., Barrish, J.O., Geerts, S., Lane, J.B., Percy, A.K., Annese, F., McNair, L., Skinner, S.A., Lee, H.-S., et al. (2012). Gastrointestinal and Nutritional Problems Occur Frequently Throughout Life in Girls and Women With Rett Syndrome: *Journal of Pediatric Gastroenterology and Nutrition* 55, 292–298.
- Munz, M., Brecht, M., and Wolfe, J. (2010). Active Touch During Shrew Prey Capture. *Frontiers in Behavioral Neuroscience* 4.
- Neul, J.L., Fang, P., Barrish, J., Lane, J., Caeg, E.B., Smith, E.O., Zoghbi, H., Percy, A., and Glaze, D.G. (2008). Specific mutations in Methyl-CpG-Binding Protein 2 confer different severity in Rett syndrome. *Neurology* 70, 1313–1321.
- Neul, J.L., Lane, J.B., Lee, H.-S., Geerts, S., Barrish, J.O., Annese, F., Baggett, L., Barnes, K., Skinner, S.A., Motil, K.J., et al. (2014). Developmental delay in Rett syndrome: data from the natural history study. *J Neurodev Disord* 6, 20.
- Neul, J.L., Benke, T.A., Marsh, E.D., Skinner, S.A., Merritt, J., Lieberman, D.N., Standridge, S., Feyma, T., Heydemann, P., Peters, S., et al. (2018). The array of clinical phenotypes of males with mutations in *Methyl-CpG binding protein 2*. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*.
- Nguyen, M.V.C., Du, F., Felice, C.A., Shan, X., Nigam, A., Mandel, G., Robinson, J.K., and Ballas, N. (2012). MeCP2 Is Critical for Maintaining Mature Neuronal Networks

and Global Brain Anatomy during Late Stages of Postnatal Brain Development and in the Mature Adult Brain. *Journal of Neuroscience* 32, 10021–10034.

Nomura, Y. (2005). Early behavior characteristics and sleep disturbance in Rett syndrome. *Brain and Development* 27, S35–S42.

Nowicka, D., Soulsby, S., Skangiel-Kramska, J., and Glazewski, S. (2009). Parvalbumin-containing neurons, perineuronal nets and experience-dependent plasticity in murine barrel cortex. *European Journal of Neuroscience* 30, 2053–2063.

O'Connor, D.H., Peron, S.P., Huber, D., and Svoboda, K. (2010). Neural Activity in Barrel Cortex Underlying Vibrissa-Based Object Localization in Mice. *Neuron* 67, 1048–1061.

Okabe, S., Nagasawa, M., Kihara, T., Kato, M., Harada, T., Koshida, N., Mogi, K., and Kikusui, T. (2013). Pup odor and ultrasonic vocalizations synergistically stimulate maternal attention in mice. *Behavioral Neuroscience* 127, 432–438.

Oohashi, T., Edamatsu, M., Bekku, Y., and Carulli, D. (2015). The hyaluronan and proteoglycan link proteins: Organizers of the brain extracellular matrix and key molecules for neuronal function and plasticity. *Experimental Neurology* 274, 134–144.

Orefice, L.L., Zimmerman, A.L., Chirila, A.M., Sleboda, S.J., Head, J.P., and Ginty, D.D. (2016). Peripheral Mechanosensory Neuron Dysfunction Underlies Tactile and Behavioral Deficits in Mouse Models of ASDs. *Cell* 166, 299–313.

Pacchiarini, N., Fox, K., and Honey, R.C. (2017). Perceptual learning with tactile stimuli in rodents: Shaping the somatosensory system. *Learning & Behavior* 45, 107–114.

Park, J.M., Hong, Y.K., Rodgers, C.C., Dahan, J.B., Schmidt, E.R., and Bruno, R.M. (2020). Deep and superficial layers of the primary somatosensory cortex are critical for whisker-based texture discrimination in mice (*Neuroscience*).

Pelka, G.J., Watson, C.M., Christodoulou, J., and Tam, P.P.L. (2005). Distinct expression profiles of *Mecp2* transcripts with different lengths of 3'UTR in the brain and visceral organs during mouse development. *Genomics* 85, 441–452.

Pesheva, P., and Probstmeier, R. (2000). The yin and yang of tenascin-R in CNS development and pathology. *Prog. Neurobiol.* 61, 465–493.

Petersen, C.C.H. (2007). The Functional Organization of the Barrel Cortex. *Neuron* 56, 339–355.

Picker, J.D., Yang, R., Ricceri, L., and Berger-Sweeney, J. (2006). An altered neonatal behavioral phenotype in *Mecp2* mutant mice: *NeuroReport* 17, 541–544.

Pizzo, R., Gurgone, A., Castroflorio, E., Amendola, E., Gross, C., Sassoè-Pognetto, M., and Giustetto, M. (2016). Lack of *Cdkl5* Disrupts the Organization of Excitatory and Inhibitory Synapses and Parvalbumin Interneurons in the Primary Visual Cortex. *Front Cell Neurosci* 10, 261.

- Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J.W., and Maffei, L. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. *Science* 298, 1248–1251.
- Pizzorusso, T., Medini, P., Landi, S., Baldini, S., Berardi, N., and Maffei, L. (2006). Structural and functional recovery from early monocular deprivation in adult rats. *Proceedings of the National Academy of Sciences* 103, 8517–8522.
- Rao, R.P., Mielke, F., Bobrov, E., and Brecht, M. (2014). Vocalization–whisking coordination and multisensory integration of social signals in rat auditory cortex. *ELife* 3.
- Reinhard, S.M., Rais, M., Afroz, S., Hanania, Y., Pendi, K., Espinoza, K., Rosenthal, R., Binder, D.K., Ethell, I.M., and Razak, K.A. (2019). Reduced perineuronal net expression in Fmr1 KO mice auditory cortex and amygdala is linked to impaired fear-associated memory. *Neurobiol Learn Mem* 164, 107042.
- Renthal, W., Boxer, L.D., Hrvatin, S., Li, E., Silberfeld, A., Nagy, M.A., Griffith, E.C., Vierbuchen, T., and Greenberg, M.E. (2018). Characterization of human mosaic Rett syndrome brain tissue by single-nucleus RNA sequencing. *Nature Neuroscience* 21, 1670–1679.
- Rett, A. (1966). [On a unusual brain atrophy syndrome in hyperammonemia in childhood]. *Wien Med Wochenschr* 116, 723–726.
- Ribeiro, M.C., and MacDonald, J.L. (2020). Sex differences in Mecp2-mutant Rett syndrome model mice and the impact of cellular mosaicism in phenotype development. *Brain Research* 1729, 146644.
- Rietveld, L., Stuss, D.P., McPhee, D., and Delaney, K.R. (2015). Genotype-specific effects of Mecp2 loss-of-function on morphology of Layer V pyramidal neurons in heterozygous female Rett syndrome model mice. *Frontiers in Cellular Neuroscience* 9.
- Robinson, L., Plano, A., Cobb, S., and Riedel, G. (2013). Long-term home cage activity scans reveal lowered exploratory behaviour in symptomatic female Rett mice. *Behavioural Brain Research* 250, 148–156.
- Samaco, R.C., McGraw, C.M., Ward, C.S., Sun, Y., Neul, J.L., and Zoghbi, H.Y. (2013). Female Mecp2^{+/-} mice display robust behavioral deficits on two different genetic backgrounds providing a framework for pre-clinical studies. *Human Molecular Genetics* 22, 96–109.
- Santos, M., Silva-Fernandes, A., Oliveira, P., Sousa, N., and Maciel, P. (2007). Evidence for abnormal early development in a mouse model of Rett syndrome. *Genes, Brain and Behavior* 6, 277–286.
- Skene, P.J., Illingworth, R.S., Webb, S., Kerr, A.R.W., James, K.D., Turner, D.J., Andrews, R., and Bird, A.P. (2010). Neuronal MeCP2 Is Expressed at Near Histone-Octamer Levels and Globally Alters the Chromatin State. *Molecular Cell* 37, 457–468.

Sofroniew, N.J., Cohen, J.D., Lee, A.K., and Svoboda, K. (2014). Natural Whisker-Guided Behavior by Head-Fixed Mice in Tactile Virtual Reality. *Journal of Neuroscience* 34, 9537–9550.

Song, C., Feodorova, Y., Guy, J., Peichl, L., Jost, K., Kimura, H., Cardoso, M., Bird, A., Leonhardt, H., Joffe, B., et al. (2014). DNA methylation reader MECP2: cell type- and differentiation stage-specific protein distribution. *Epigenetics & Chromatin* 7, 17.

Sorg, B.A., Berretta, S., Blacktop, J.M., Fawcett, J.W., Kitagawa, H., Kwok, J.C.F., and Miquel, M. (2016). Casting a Wide Net: Role of Perineuronal Nets in Neural Plasticity. *The Journal of Neuroscience* 36, 11459–11468.

Sreenivasan, V., Karmakar, K., Rijli, F.M., and Petersen, C.C.H. (2015). Parallel pathways from motor and somatosensory cortex for controlling whisker movements in mice. *European Journal of Neuroscience* 41, 354–367.

Stearns, N.A., Schaevitz, L.R., Bowling, H., Nag, N., Berger, U.V., and Berger-Sweeney, J. (2007). Behavioral and anatomical abnormalities in *Mecp2* mutant mice: A model for Rett syndrome. *Neuroscience* 146, 907–921.

Stefanelli, G., Gandaglia, A., Costa, M., Cheema, M.S., Di Marino, D., Barbiero, I., Kilstrup-Nielsen, C., Ausió, J., and Landsberger, N. (2016). Brain phosphorylation of MeCP2 at serine 164 is developmentally regulated and globally alters its chromatin association. *Sci Rep* 6, 28295.

Stern, J.M. (1990). Multisensory regulation of maternal behavior and masculine sexual behavior: A revised view. *Neuroscience & Biobehavioral Reviews* 14, 183–200.

Stern, J.M., and Kolunie, J.M. (1989). Perioral anesthesia disrupts maternal behavior during early lactation in Long-Evans rats. *Behav. Neural Biol.* 52, 20–38.

Stolzenberg, D.S., and Champagne, F.A. (2016). Hormonal and non-hormonal bases of maternal behavior: The role of experience and epigenetic mechanisms. *Horm Behav* 77, 204–210.

Stolzenberg, D.S., and Rissman, E.F. (2011). Oestrogen-independent, experience-induced maternal behaviour in female mice. *J. Neuroendocrinol.* 23, 345–354.

Sugiyama, S., Di Nardo, A.A., Aizawa, S., Matsuo, I., Volovitch, M., Prochiantz, A., and Hensch, T.K. (2008). Experience-Dependent Transfer of *Otx2* Homeoprotein into the Visual Cortex Activates Postnatal Plasticity. *Cell* 134, 508–520.

Suttkus, A., Rohn, S., Weigel, S., Glöckner, P., Arendt, T., and Morawski, M. (2017). Aggrecan, link protein and tenascin-R are essential components of the perineuronal net to protect neurons against iron-induced oxidative stress. *Cell Death & Disease* 5, e1119–e1119.

Tarquinio, D.C., Hou, W., Berg, A., Kaufmann, W.E., Lane, J.B., Skinner, S.A., Motil, K.J., Neul, J.L., Percy, A.K., and Glaze, D.G. (2017). Longitudinal course of epilepsy in Rett syndrome and related disorders. *Brain* 140, 306–318.

Tarquinio, D.C., Hou, W., Neul, J.L., Berkmen, G.K., Drummond, J., Aronoff, E., Harris, J., Lane, J.B., Kaufmann, W.E., Motil, K.J., et al. (2018). The course of awake

breathing disturbances across the lifespan in Rett syndrome. *Brain and Development* 40, 515–529.

Ueno, H., Suemitsu, S., Okamoto, M., Matsumoto, Y., and Ishihara, T. (2017). Sensory experience-dependent formation of perineuronal nets and expression of Cat-315 immunoreactive components in the mouse somatosensory cortex. *Neuroscience* 355, 161–174.

Ueno, H., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Matsumoto, Y., Aoki, S., Okamoto, M., and Ishihara, T. (2018a). Hyaluronic acid is present on specific perineuronal nets in the mouse cerebral cortex. *Brain Research* 1698, 139–150.

Ueno, H., Takao, K., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Okamoto, M., Aoki, S., and Ishihara, T. (2018b). Age-dependent and region-specific alteration of parvalbumin neurons and perineuronal nets in the mouse cerebral cortex. *Neurochemistry International* 112, 59–70.

Vignoli, A., Fabio, R.A., La Briola, F., Giannatiempo, S., Antonietti, A., Maggiolini, S., and Canevini, M.P. (2010). Correlations between neurophysiological, behavioral, and cognitive function in Rett syndrome. *Epilepsy & Behavior* 17, 489–496.

Villard, L. (2007). MECP2 mutations in males. *Journal of Medical Genetics* 44, 417–423.

Vo, T., Carulli, D., Ehlert, E.M.E., Kwok, J.C.F., Dick, G., Mecollari, V., Moloney, E.B., Neufeld, G., de Winter, F., Fawcett, J.W., et al. (2013). The chemorepulsive axon guidance protein semaphorin3A is a constituent of perineuronal nets in the adult rodent brain. *Molecular and Cellular Neuroscience* 56, 186–200.

Wallace, H., and Fox, K. (1999). Local cortical interactions determine the form of cortical plasticity. *J Neurobiol* 41, 58–63.

Walsh, C.J., Fleming, A.S., Lee, A., and Magnusson, J.E. (1996). The effects of olfactory and somatosensory desensitization on Fos-like immunoreactivity in the brains of pup-exposed postpartum rats. *Behavioral Neuroscience* 110, 134–153.

Weiss, J., Pyrski, M., Jacobi, E., Bufe, B., Willnecker, V., Schick, B., Zizzari, P., Gossage, S.J., Greer, C.A., Leinders-Zufall, T., et al. (2011). Loss-of-function mutations in sodium channel Nav1.7 cause anosmia. *Nature* 472, 186–190.

Welker, E., Soriano, E., Dörfl, J., and Van der Loos, H. (1989). Plasticity in the barrel cortex of the adult mouse: transient increase of GAD-immunoreactivity following sensory stimulation. *Exp Brain Res* 78, 659–664.

Welker, E., Rao, S.B., Dörfl, J., Melzer, P., and van der Loos, H. (1992). Plasticity in the barrel cortex of the adult mouse: effects of chronic stimulation upon deoxyglucose uptake in the behaving animal. *J. Neurosci.* 12, 153–170.

Welker, W., Sanderson, K.J., and Shambes, G.M. (1984). Patterns of afferent projections to transitional zones in the somatic sensorimotor cerebral cortex of albino rats. *Brain Research* 292, 261–267.

- Woolsey, T.A., and Van der Loos, H. (1970). The structural organization of layer IV in the somatosensory region (S I) of mouse cerebral cortex. *Brain Research* 17, 205–242.
- Wu, H., Luo, J., Yu, H., Rattner, A., Mo, A., Wang, Y., Smallwood, P.M., Erlanger, B., Wheelan, S.J., and Nathans, J. (2014). Cellular Resolution Maps of X Chromosome Inactivation: Implications for Neural Development, Function, and Disease. *Neuron* 81, 103–119.
- Wu, H.-P.P., Ioffe, J.C., Iverson, M.M., Boon, J.M., and Dyck, R.H. (2013). Novel, whisker-dependent texture discrimination task for mice. *Behav. Brain Res.* 237, 238–242.
- Xerri, C., Stern, J., and Merzenich, M. (1994). Alterations of the cortical representation of the rat ventrum induced by nursing behavior. *The Journal of Neuroscience* 14, 1710–1721.
- Xerri, C., Bourgeon, S., and Coq, J.-O. (2005). Perceptual context-dependent remodeling of the forepaw map in the SI cortex of rats trained on tactile discrimination. *Behavioural Brain Research* 162, 207–221.
- Xinhua Bao, Shengling Jiang, Fuying Song, Hong Pan, Meirong Li, and Wu, X.-R. (2008). X Chromosome Inactivation in Rett Syndrome and Its Correlations With MeCP2 Mutations and Phenotype. *J Child Neurol* 23, 22–25.
- Zhou, Z., Hong, E.J., Cohen, S., Zhao, W., Ho, H.H., Schmidt, L., Chen, W.G., Lin, Y., Savner, E., Griffith, E.C., et al. (2006). Brain-Specific Phosphorylation of MeCP2 Regulates Activity-Dependent Bdnf Transcription, Dendritic Growth, and Spine Maturation. *Neuron* 52, 255–269.

Appendix

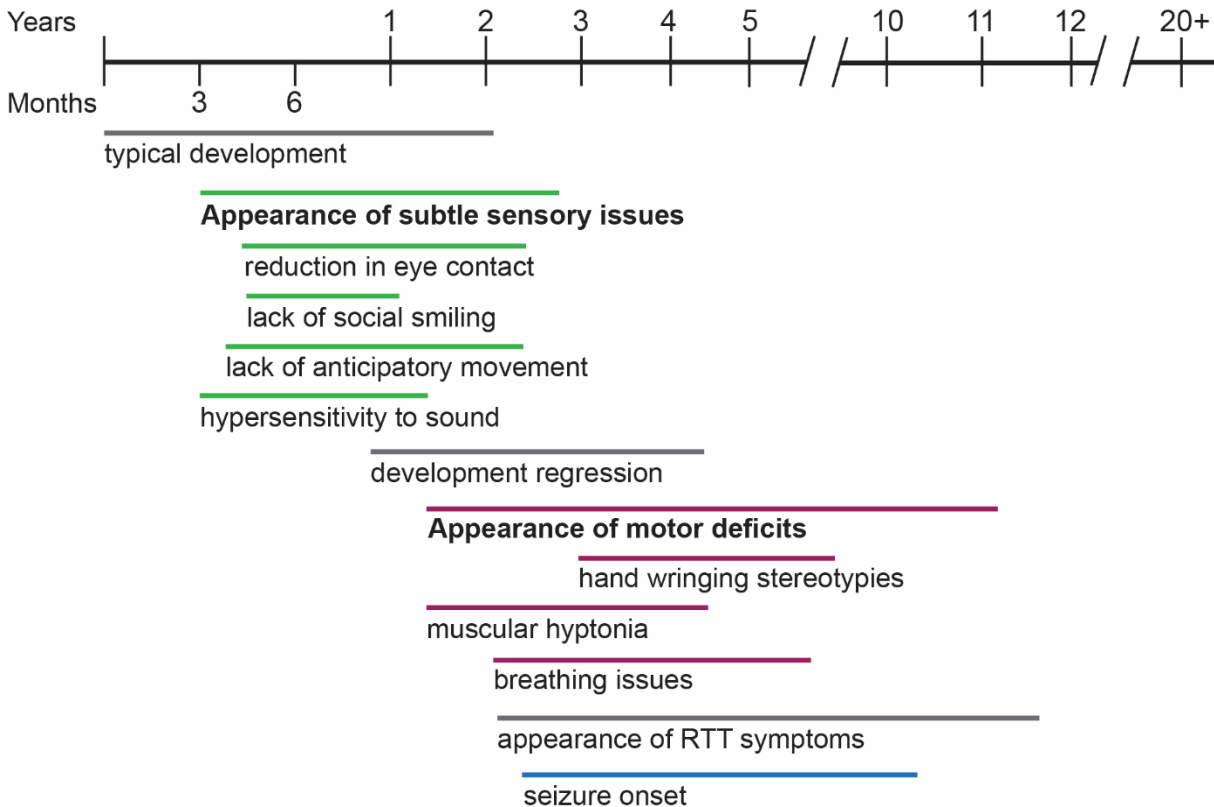


Figure 1. Timeline of symptom onset in female Rett Syndrome patients.

Age is indicated at the top in months (bottom) and years (top). Lines indicate the period in which the corresponding stage or symptom appears. Gray lines indicate the traditionally viewed characteristic development of Rett Syndrome symptoms. Green lines indicate the appearance of sensory issues during the 'typical period of development'. Magenta lines indicate motor symptom onset, which correlated with syndromic onset. The gray lines are modified from Chahrour and Zoghbi, 2007, all the other information is collected from Buchanan et al., 2019; Djukic and Valicenti McDermott, 2012; FitzGerald et al., 1990; Jian et al., 2006; Killian et al., 2017; Motil et al., 2012; Neul et al., 2014; Nomura, 2005; Tarquinio et al., 2017, 2018; Vignoli et al., 2010

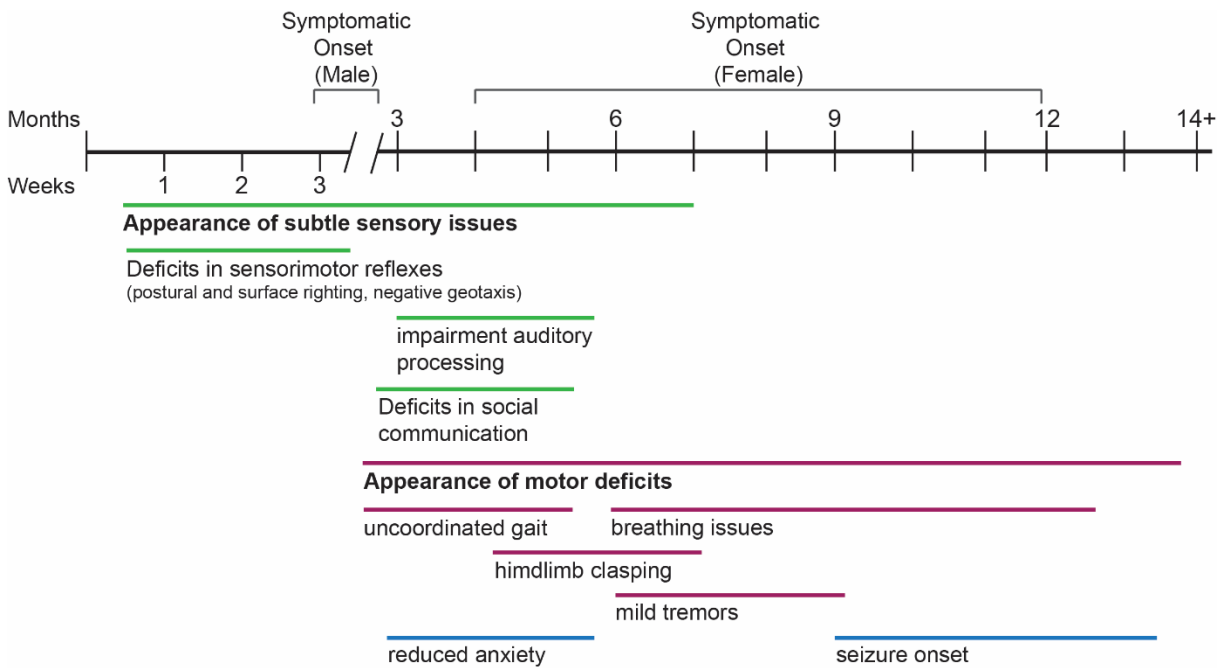


Figure 2. Development of and RTT symptom progression in the *Mecp2^{Het}* a female mouse model of RTT

The female mouse model is characterized by late onset of RTT symptomatic features (~16 weeks old) in comparison to the male mouse model, which becomes symptomatic around 3-4 weeks of age. Symptom onset is preceded by sensory deficits (green lines) and the appearance of motor dysfunction (magenta lines). Data collected from Guy et al., 2001; Krishnan et al., 2015, 2017; Lau et al., 2020; Picker et al., 2006; Robinson et al., 2013; Samaco et al., 2013; Santos et al., 2007; Stearns et al., 2007

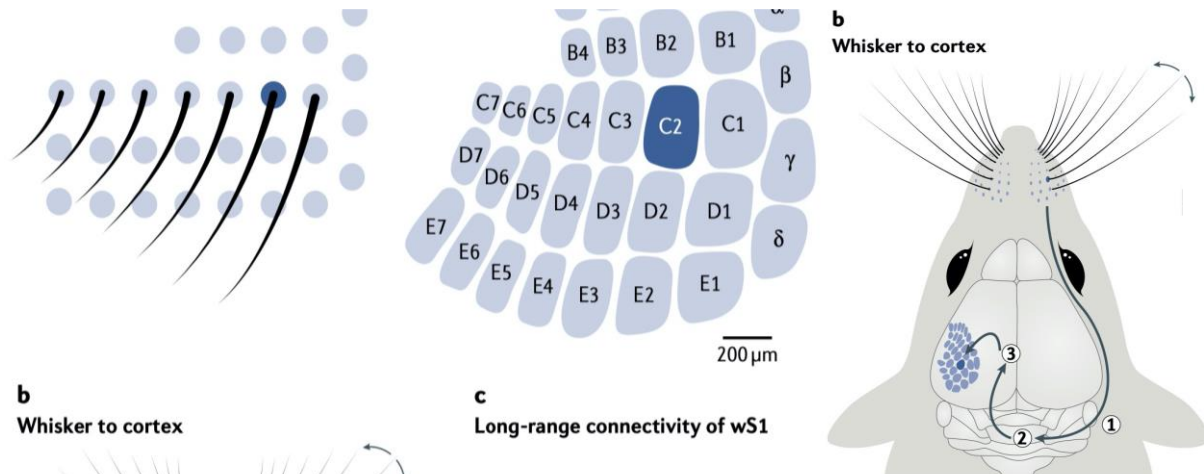


Figure 3. Connectivity of the Primary Somatosensory Cortex Barrel Field Subregion.

The barrel cortex has been well-studied as an experience-dependent plasticity model due to the plasticity of the barreloid structures in response to whisker activity (A). Each whisker on the mystacial pad (A, left) sends information to a specific barreloid in the SS1 (A, right). B. Information from the whiskers travels from the mystacial pad via the trigeminal ganglia (1) to the brainstem (2) through the thalamus (3) and into the barrel cortex. Adapted from Petersen, C.C.H. *Sensorimotor processing in the rodent barrel cortex*. 2019.

CHAPTER I
QUALITATIVE CHARACTERIZATION OF WHOLE BRAIN
PERINEURONAL NET EXPRESSION AFTER MATERNAL
EXPERIENCE IN A FEMALE MOUSE MODEL OF RETT SYNDROME

Abstract

The pup retrieval paradigm is a multisensory social component of learned maternal behavior, where the adult locates scattered pups and bring them back to the nest. Virgin female mice, with no prior maternal experience (Naïve), efficiently retrieve pups after being co-housed with a first-time dam and her pups (then termed surrogates 'Sur'), inducing plasticity changes in the Sur auditory cortex. As such, this paradigm can be used to study adult experience-dependent plasticity mechanisms in a complex multisensory behavior. Experience-dependent plasticity in the auditory cortex of the adult female mouse model of Rett Syndrome (RTT) was dysregulated during the pup retrieval paradigm, causing inefficient retrieval. RTT is a neurological disorder caused by mutations in methyl-CpG-binding protein 2 (*MECP2*), an activity-dependent gene expression regulator. Inefficient pup retrieval behavior caused dysregulation in cortical inhibitory networks, as marked by increased parvalbumin (PV) and perineuronal net (PNN) expression in adult *Mecp2*^{Het} (Het) auditory cortex. PNNs are extracellular matrix structures surrounding PV+ GABAergic neurons in the cortex after a new experience, marking restricted plasticity in the brain. When PNNs were removed from the auditory cortex, the SurHet retrieval efficiency improved, but not significantly when compared to SurWT. Partial rescue of the retrieval phenotype suggested that plasticity in another region involved in this paradigm is dysregulated in the SurHet. To identify other regions of the SurHet brain that were undergoing dysregulated plasticity mechanisms, we did a whole brain qualitative comparison analysis of alterations in PNN expression between the NaïveWT, NaïveHet, SurWT and SurHet brains. This descriptive analysis identified PNN changes in many regions after behavior in both the WT and Het brains with known involvement in maternal behaviors, multisensory integration, and learning. From this analysis, six regions were picked for an exploratory whole brain quantitative PNN expression analysis. We report that the WT brain undergoes hemisphere-specific remodeling of PNN expression after behavior within the motor cortex. We also found increased PNN expression in the primary somatosensory cortex of the SurHet, suggesting that this region may also be contributing to inefficient pup retrieval by the SurHet. Here, we have identified and

eliminated multiple regions of interest for more in-depth studies on adult experience-dependent plasticity mechanisms in SurHet brain based on high-intensity PNN expression.

Introduction

Maternal behaviors consist of stereotypic components such as nursing, pup grooming, nest building, and pup retrieval, which all revolve around the care and maintenance of pups. The onset of these maternal behaviors is dependent on the processing of auditory, olfactory, and somatosensory cues from pups (Beach and Jaynes, 1956; Lonstein et al., 2015; Stern, 1990). Virgin female mice (Naïve) are able to learn aspects of maternal behavior, such as pup grooming and pup retrieval, from interacting with the pups and the mother (Alsina-Llanes et al., 2015). When these Naïve females are co-housed with a first-time mother, then termed Surrogates (Sur), they become increasingly efficient in pup retrieval and other pup-directed behaviors (Alsina-Llanes et al., 2015; Cohen et al., 2011). Auditory cues from pups induce plasticity within the auditory cortex of these surrogates (Cohen et al., 2011; Galindo-Leon et al., 2009), identifying the pup retrieval assay as a way to study adult experience-dependent plasticity mechanisms within an etiologically relevant behavior.

Previously, we showed that adult experience-dependent plasticity was dysregulated in the auditory cortex of an adult female mouse model of Rett Syndrome, resulting in inefficient pup retrieval (Krishnan et al., 2017). Rett Syndrome is a neurological disorder caused by mutations in methyl CpG-binding protein 2 (*MECP2*) (Amir et al., 1999). *MECP2* regulates neuronal chromatin architecture and gene transcription in response to neural activity and experience throughout development and into adulthood (Bedogni et al., 2016; Ebert et al., 2013; Kishi and Macklis, 2004; Skene et al., 2010; Stefanelli et al., 2016; Zhou et al., 2006). Based on the known functions of *MECP2*, we hypothesize that *MECP2* regulates experience-dependent plasticity in specific neural circuits during windows of enhanced sensory and social experience throughout life, like the pup retrieval paradigm. Surrogate *Mecp2*^{Het} (SurHet) were inefficient at the pup retrieval paradigm due to dysregulated inhibitory

networks and an increased expression of perineuronal nets within the auditory cortex (Krishnan et al., 2017; Lau et al., 2020).

Perineuronal nets (PNNs) are extracellular matrix structures that form around the soma and proximal dendrites of PV+ GABAergic interneurons, solidifying newly formed synaptic connections on the neuron after heightened periods of plasticity (Cattaui et al., 2018; Gogolla et al., 2009; Hartig et al., 1992; Miyata and Kitagawa, 2017; Murthy et al., 2019; Sigal et al., 2019). During development, PNN expression, along with inhibitory markers like PV, increases with the maturation of the visual cortex, correlating with the termination of the visual critical period (Hartig et al., 1992; Hou et al., 2017). In the adult cortex, mature PNNs are thought to be stable structures that regulate experience-dependent plasticity and play roles in long term memory retention (Carstens et al., 2016; Cattaui et al., 2018; Gogolla et al., 2009; Krishnan et al., 2015, 2017; Thompson et al., 2018).

PNN structures can be degraded through the injection of chondroitinase ABC, which results in the reactivation of synaptic plasticity within the affected region (Banerjee et al., 2017; Gogolla et al., 2009; Kochlamazashvili et al., 2010; Pizzorusso et al., 2002; Thompson et al., 2018). When the formation of PNNs is dysregulated, as is seen in mouse models for differing neurological disorders, the removal of PNNs can improve the behavioral readout of the experiment (Berretta et al., 2015; Krishnan et al., 2017; Pizzo et al., 2016; Reinhard et al., 2019). When PNNs were enzymatically degraded from the auditory cortex of the SurHet, there was a partial rescue of the pup retrieval behavior (Krishnan et al., 2017). The SurHets that had been injected with chondroitinase ABC performed more efficiently at the pup gathering paradigm than they had before the PNNs were degraded. However, there was only a partial rescue of the behavior, as they did not perform as efficiently as the SurWT (Krishnan et al., 2017). This suggested that another brain region or circuit may be involved in this behavior.

The pup retrieval assay relies on the integration of auditory, olfactory, and somatosensory information for efficient execution of the behavior, and there are many known regions of the brain that are important for not only pup retrieval, but other pup-

directed behaviors that are occurring during co-housing (reviewed in Beach and Jaynes, 1956; Kohl et al., 2017; Lonstein et al., 2015; Stern, 1990). Here, we did a whole brain qualitative analysis of PNN expression changes within brain regions across the NaïveWT, NaïveHet, SurWT and SurHet conditions of the pup retrieval experiments. We observed that many regions with known roles in maternal behavior undergo alterations in PNN expression, often in a hemisphere-specific manner. PNN expression changes within a subset of these brain regions were quantified in a whole brain and hemisphere-specific manner. We identified dynamic PNN expression changes in the WT animals in the motor cortex suggesting dynamic hemisphere-specific remodeling of PNN expression in the adult brain after pup gathering. We also found that SurHet had significantly more PNNs in the primary somatosensory cortex than the SurWT did, a change that was present in the right hemisphere but not the left.

During the analysis of the primary somatosensory cortex, we found a distinct difference in the quantity and intensity of PNNs between the medial and lateral sections, regardless of condition. This could suggest a specific circuit that is being dysregulated within the SS1 of the Het, contributing to poor pup gathering behavior. As this was an exploratory analysis, we suggest that future quantification be done in a somatosensory cortex subregion-specific manner to highlight any specific functional regions/circuits that are exhibiting maladaptive PNN formation in the Het.

Methods

Animals

All animals raised on a 12/12-hour light/dark cycle and received food ad libitum. All animals were between 10-12 weeks old at the time of behavior. Animals used were CBA/CaJ, *Mecp2*^{Het} (C57BL/6, B6.129P2(C)-*Mecp2*^{tm1.1Bird/J}) and *Mecp2*^{WT}.

Pup gathering behavior

The pup gathering behavior was performed similarly to Krishnan et al., 2017. Two Naïve females, one *Mecp2*^{WT} and one *Mecp2*^{Het}, were co-housed with a pregnant CBA/CaJ female 3-5 days before birth; these Naïve females are then termed 'surrogate' (Sur). Once the pups are born, the gathering behavior is conducted on post-

natal day 0 (P0), P3 and P5 for each adult separately. First, the adult is placed in the home cage with the pups for 5 minutes (*habituation*) before the pups are removed for 2 minutes (*isolation*). The pups are then placed back into the cage, scattered into the corners and the center of the cage (*retrieval*). The adult was then recorded for 5 minutes. After the behavior was completed, all mice were returned to the home cage. These were performed in a sound-proof dark box during the light cycle (between 10:30 AM and 4 PM). Surrogates and age-matched Naïves were perfused after immediately P5 behavior. Naïves did not experience any new behavior.

Immunohistochemistry

The brains were extracted and stored in 4% paraformaldehyde/PBS solution overnight, which was exchanged for PBS for storage until processing. Before sectioning, samples were incubated overnight in a 30% sucrose/PBS solution. Brains were cut in half to separate the left and right hemispheres before being sectioned at 50 μ m in a sagittal orientation using a vibratome. Free-floating sections were blocked using a 10% normal goat serum and 0.5% Triton X-100 solution at room temperature (RT) for 3 hours before a 4-hour primary incubation step with biotin-conjugated WFA lectin (1:500; Sigma-Aldrich) in a 5% normal goat serum and 0.25% Triton X-100 solution. The sections were incubated in a secondary solution (5% normal goat serum and 0.25% Triton X-100) containing streptavidin-conjugated Alexa Fluor 488 secondary antibodies (1:1000; Invitrogen) for 2 hours. Sections were then counterstained with the nuclear marker, DAPI (1:1000) for 5 minutes. The free-floating sections were mounted onto slides using Fluoromount-G (Southern Biotech).

Descriptive comparison imaging and analysis

For the qualitative PNN expression comparison analysis, two whole cohorts (1 cohort = NW, NH, SW, SH) were imaged using the epifluorescent Keyence microscope (Keyence BZ-X710; Keyence Corp.). Each section was imaged using 20x magnification, then the images were stitched together using the BZ-X Analyzer software (Keyence). The sections were assigned map numbers based on bregma location in the Paxinos and Franklin's *The Mouse Brain* atlas (Paxinos and Franklin, 2013). For comparative analysis, sections from each condition within the same map

number were compared to one another and regions that looked as if there was a change in PNN expression (i.e. higher/lower expression) were noted (the result being Tables 1.1-4). The left and right hemispheres were kept separate during the analysis. An example of this is shown in Figure 1.1.

PNN quantification imaging and analysis

For quantitative PNN expression analysis across multiple regions, whole region epifluorescent projection images were acquired using the Keyence microscope (Keyence BZ-X710; Keyence Corp.). Three cohorts were used in this exploratory analysis. Each z-series was imaged using 20x magnification, in 1 μ m steps, then the images were stitched together and compressed using the Keyence stitching software. Imaging settings for each region of each cohort was based on the SH. To reduce overexposure, the exposure time was set by finding the point at which the first saturated pixel appeared in the sample, then reducing the exposure by one unit (based on the software). This was done for all SH left hemisphere sections then average exposure time was used to image all sections within that cohort.

High-intensity PNN quantification was done using ImageJ software (Schneider et al., 2012). The stitched image was opened in ImageJ, then the corresponding sagittal map template from Paxinos and Franklin's *The Mouse Brain* atlas (Paxinos and Franklin, 2013) was overlaid onto the micrograph, the specific landmarks for this differed with the region being analyzed, however the procedure is outlined in Mapping and Counting High-Intensity Perineuronal Nets in the Somatosensory Cortex (<https://dx.doi.org/10.17504/protocols.io.bcf8itrw>) (Layo et al., 2020). High-intensity PNNs were quantified by maxing out the contrast of the image and manually marking the signals that were 80% of the original PNN and higher. Statistical analysis and graphs were generated using GraphPad Prism.

Results

Whole brain descriptive analysis of PNN expression

Through a whole brain qualitative analysis of alterations in PNN expression in the adult WT and Het brain, pre- and post- learned maternal behavior, we identified

many different brain regions that could be involved in learned maternal behavior (WT) as well as regions that could be undergoing maladaptive plasticity mechanisms during the behavior (Het). We separated the qualitative analysis into subcortical areas, amygdala, hippocampal and cortical regions (Tables 1.1-4). The pup gathering assay is a multisensory paradigm, so we surveyed regions that may be involved in sensory integration, learning and maternal behavior.

During this qualitative analysis, we noticed how different PNN structures appeared between regions of the brain. Within the cortex, PNNs had the reticular-like structure that is normally shown in review papers and considered a mature PNN structure (Figure 1.2, A-C; Figure 1.3 C-E; Figure 1.4, C & D) (Miyata and Kitagawa, 2017; Ueno et al., 2017). Within cortical regions, we noticed that regions like the visual, somatosensory, and entorhinal cortices (Figure 1.2, A & C; Figure 1.4 D) had more densely packed PNNs than the insular, piriform, or motor cortices (Figure 1.2, B & G; Figure 1.3 C-E). Additionally, PNNs in the piriform cortex had more of a diffuse structure, seen in developing immature PNNs (Ueno et al., 2018), when compared to the other cortical regions and had to be imaged at a much higher exposure setting than the rest of the cortex (Figure 1.2, G)

In non-cortical regions, the PNNs were much more sparsely located and not nearly as bright as the cortex, so these regions were imaged at higher exposure settings (Figure 1.2, D-H). PNNs in the hippocampus had an interesting pattern, as most of the regions in this area had sparse PNN expression, but the PNNs were often seen on the periphery of the granular layers (as modeled by the darker < shape in the dentate gyrus, Figure 1.2 D). The exception to this was the CA2, which has distinct PNN structures on the periphery and inside the granular layer on top of what looks like a hazy background due to diffuse WFA+ signal (Figure 1.2 E). We saw a similar haziness in the piriform cortex and thought it may be a staining artifact, however it remains consistent within and across conditions as well as between cohorts. In subcortical regions, PNNs appear to be surrounding more of the proximal dendrites and axons of the neurons than in the cortex, as shown in the olfactory tubercle and nucleus of the lateral olfactory tract (Figure 1.2 F & H). As both regions are transition

zones for olfactory information, PNN components may be surrounding more of the dendrites and axons to prevent modulation of this olfactory information. While differences in PNN structure and quantity between regions was noticed in the descriptive analysis, we also identified many regions of the brain that exhibited alterations in PNN expression between 1 or more of the condition comparisons outlined in Tables 1.1-4.

The primary somatosensory cortex seemed to be undergoing many different alterations in PNN expression, with the NaïveHet (Table 1.3, column 1) or SurHet (Table 1.3, columns 2 & 4) consistently being higher. Somatosensation through tactile feedback from pups is particularly important for the development of maternal behavior; pup gathering in particular (Stern, 1996). When tactile input from the ventral trunk or the face is restricted, pup care, including pup retrieval, is disrupted (Kenyon et al., 1981, 1983; Morgan et al., 1992; Stern and Kolunje, 1989; Walsh et al., 1996). Somatosensory input induces activity within the periaqueductal gray, which when lesioned disrupts pup retrieval, though we did not see maternal related PNN changes in this region (Table 1.4) (Lonstein and Stern, 1997, 1999). We also saw that PNN expression may be increased in the Hets within the insular cortex, a region that has been implicated in multisensory integration (Table 1.3, column 2 & 4; Figure 1.2 B) (Gogolla et al., 2014). This study actually looked at *Mecp2*-null males who showed little activity in the insular cortex when compared to WT mice during periods of time when they are receiving both auditory and tactile input (Gogolla et al., 2014).

Other senses important to learned maternal behavior are audition and olfaction. We have already shown that atypical PNN expression in the SurHet auditory cortex disrupts pup gathering (Krishnan et al., 2017). Here we note maternal behavior-based changes in PNN expression within the piriform cortex (Table 1.3, column 4), which is involved in olfaction (Figure 1.2, G) (Stettler and Axel, 2009; Ueno et al., 2019; Walsh et al., 1996). During pup retrieval, pup olfactory cues are integrated with pup ultrasonic vocalization calls in the auditory cortex (Cohen et al., 2011). The integration of this information also induces activity in the central amygdala and basolateral amygdala, as measured by c-Fos staining (Calamandrei and Keverne, 1994; Okabe et al., 2013).

Both regions appear to increase in PNN expression in the SurHet (Table 1.1, column 4). Within the olfactory system, we also noted Het-based increases in PNNs within the olfactory tubercle, which connects the piriform cortex and olfactory bulb to the basolateral amygdala (Table 1.4, column 1 & 4; Figure 1.2 F). When Naïve females are exposed to pups, neurons in these regions, along with the medial entorhinal cortex, are activated, as measured through c-Fos staining (Calamandrei and Keverne, 1994). The entorhinal cortex sends odor sensory information from the piriform cortex and lateral nucleus of the olfactory tract to the hippocampus (Table 1.3, column 2 & 4; Figure 1.2 C) (Biella and de Curtis, 2000).

Of the changes that were observed between conditions, many of them could indicate circuitry involved in learned maternal behavior needed for efficient pup gathering (WT) or circuitry that is being dysregulated (Het) causing inefficient pup gathering. The multisensory integration examples above (tactile-auditory and auditory-olfactory) show that there are multiple regions that could be explored in more depth to understand the experience-dependent plasticity underlying this behavior. Together, this analysis shows that PNN expression does not change globally with maternal behavior in the SurWT or SurHet, suggesting that there are more specific pathways undergoing changes in response to this behavior. We identified six regions of the brain for exploratory PNN quantification: the dentate gyrus, motor, somatosensory, entorhinal, insular, and visual cortices.

PNN expression increased in WT motor cortex after behavior

The motor cortex has not specifically been implicated in the circuitry of learned maternal behavior. However, the pup gathering behavior does involve motor coordination in the context of following tactile, olfactory, and auditory cues in search of the pups while they are outside of the nest. The motor cortex may play an important role in the execution of multisensory integration circuit for pup-directed movements in this behavior. Through whole cortex analysis, we found that there were no significant differences in PNN expression between the conditions when the hemisphere counts were combined (Figure 1.3, A). When the hemispheres were divided into the left and right hemisphere, we observed multiple changes in PNN expression (Figure 1.3, B).

In the left hemisphere, we found that the NaïveHet had significantly more PNNs than the NaïveWT; though this may be due to the uneven number of sections in this analysis and not biologically significant as it was not seen in the right hemisphere (Figure 1.3, B). In the left and right hemispheres, there are significant alterations in PNN expression after behavior in the WT animals (purple, Figure 1.3, B). There is a significant increase of PNNs in the SurWT when compared to the NaïveWT in the left hemisphere (Figure 1.3, B-D), however the opposite is seen in the right hemisphere (Figure 1.3 B). Within the SurWT brain, the left hemisphere has significantly more PNNs than the right hemisphere, showing a hemisphere-specific alteration in PNN expression within condition (Figure 1.3, B, D, E). As we do not see this hemisphere bias in the NaïveWT, these results suggest that experience-dependent plasticity mechanisms in the motor cortex are affected in a hemisphere-specific manner during normal learning of this behavior.

The motor cortex also plays a role in sensorimotor integration, which may explain changes seen in the WT brain. The motor cortex controls the active movement of the whiskers and tactile information from the whiskers is processed in the primary somatosensory cortex (Sreenivasan et al., 2015; Welker et al., 1984). While this has not been studied within the context of learned maternal behavior, the motor cortex does project to the somatosensory cortex and modulate activity in that region (Lee et al., 2013; Veinante and Deschênes, 2003). Our behavior is conducted in the dark and active sensing through the whiskers is one of the main sensory systems that the mice use to navigate their environment, in this case the cage when locating the pups (Arkley et al., 2014; Grant et al., 2009). As more is known about the role of somatosensation in learned maternal behaviors, we chose to quantify PNNs within this region in response to maternal behavior.

SurHet has more PNNs in primary somatosensory cortex compared to SurWT

The primary somatosensory cortex processes tactile input from all over the body (Feldman and Brecht, 2005). While it has not been specifically studied in the context of learned maternal behavior, it has been established that tactile sensory cues are important for the onset of pup-directed behaviors, like nursing and pup gathering

(reviewed in Beach and Jaynes, 1956; Stern, 1990, 1996). Many early studies deprived rats of sensory stimulation from either the perioral area (snout and whiskers) through lidocaine injections or the ventral trunk through ventral wraps. Sensory deprivation of the perioral area or ventral trunk delayed the onset of pup retrieval behavior (Kenyon et al., 1981; Morgan et al., 1992). When ventral trunk and perioral stimulation were both removed, pup gathering was significantly disrupted (Morgan et al., 1992). In Naïve female mice, physical sensitization to pups quickly induced pup-directed behaviors (Alsina-Llanes et al., 2015).

In our analysis of the primary somatosensory cortex, we found that the SurHet had increased expression of PNNs when compared to the SurWT (Figure 1.4, A, C, D). This is similar to the result seen in the auditory cortex of the SurHet, potentially implicating increased PNNs in the primary somatosensory cortex to the inefficient pup gathering behavior seen in the SurHet (Krishnan et al., 2017). This increase was isolated to the right hemisphere of the brain, suggesting hemispheric alterations may be occurring in the Het as a result of this behavior (Figure 1.4, B).

PNN intensity increases along the medial – lateral axis in the primary somatosensory cortex

The primary somatosensory cortex covers ~3 mm of each hemisphere. During analysis, we noticed that the more lateral sections had higher numbers of PNNs, regardless of condition (Figure 1.5). The primary somatosensory cortex is composed of many different functional subregions, which receive tactile information from specific parts of the body (reviewed in Brecht, 2017). The Paxinos and Franklin's *The Mouse Brain* atlas (Paxinos and Franklin, 2013), which was used to map these sections, has each of these subregions indicated in the map templates (Figure 1.1, E). The medial sections contain subregions that relate to tactile input from the trunk, hind/forelimb, and shoulder areas, whereas the lateral sections contain facial subregions, like the upper lip, jaw, and barrel field (whiskers). This suggests that PNNs are more prevalent in the lateral primary somatosensory cortex regions, compared to the medial ones.

When imaging this region, the exposure time was based on the average exposure setting between all somatosensory sections in the left hemisphere of the

SurHet. The same exposure time that reduced overexposure of the lateral sections, resulted in very low PNN numbers in the medial section (Figure 1.6, top row). When the best setting for the medial section is used, the lateral section is so overexposed that quantification of the high-intensity PNNs were not possible (Figure 1.6, bottom row). In order to quantify PNNs throughout the entire medial to lateral axis of the primary somatosensory cortex, we suggest splitting the hemisphere into lateral and medial sections based on PNN intensity and the subregions, then imaging and quantifying the two groups separately.

Alterations in PNN expression do not occur globally throughout the WT or Het brain after pup gathering experience

Based on our qualitative analysis (Tables 1.1-4), it looks as if many regions change in the Het as a result of this behavior. We found that, even though the following regions appeared to have alterations in PNN expression in the qualitative analysis, they did not once a quantitative analysis was done. We show that the insular cortex, which has been implicated in tactile and auditory sensory integration (Gogolla et al., 2014), did not exhibit any significant alterations in PNN expression between conditions (Figure 1.7, A). There is a noticeable increase of PNNs after behavior in the Het compared to the WT, however due to low number of SurWT sections it is hard to say if that would remain after the addition of more sections. Similarly, there were no significant differences between conditions, in the combined or split hemisphere analysis in the visual cortex (Figure 1.7, B). We then quantified PNNs in the dentate gyrus and caudomedial entorhinal cortex, both regions better known for memory retention and learning, where PNNs have been shown to play an important role (Biella and de Curtis, 2000; Cattaud et al., 2018; Mayer et al., 2005). In both regions, we found that there were no changes in PNN expression across conditions (Figure 1.7, C & D). Together, these results show that the novel pup gathering behavior does not induce measurable alterations in PNN expression in all regions of the SurWT or SurHet brain.

Discussion

The purpose of this exploratory study was to identify brain regions that exhibited alterations in PNN expression within the SurHet brain, as compared to the SurWT and NaïveHet, which could be contributing to the inefficient pup gathering behavior. Through the descriptive analysis (Tables 1.1-4) we identified many candidate regions for quantitative PNN analysis as a result of maternal behavior (Tables 1.1-4, column 3 & 4) as well as differences in PNN expression between genotypes in the different conditions (Tables 1.1-4, column 1 & 2). From this, we picked six regions to analyze through manual, whole brain quantification of PNNs: the dentate gyrus, motor, somatosensory, entorhinal, insular, and visual cortices. We showed that 1) not all regions of the brain undergo measurable changes in PNN expression in the WT or Het, 2) the WT undergoes alterations in PNN expression as a result of this behavior and 3) the primary somatosensory cortex shows increased PNN expression in the SurHet compared to SurWT after behavior.

In general, this was an exploratory analysis with a low number of cohorts to identify changes in PNN expression across these conditions (n= 3 cohorts, 12 total animals). Other PNN characterization papers use similar numbers of animals, however they do not do whole brain characterization studies like we have here, so the number of total sections used in the analysis are much lower (Ueno et al., 2018, 2019). As a result of our whole brain method, we identified a medial-lateral axis difference in the expression of high-intensity PNNs within the primary somatosensory cortex which had not been noted in other characteristic studies of the region (Ueno et al., 2017, 2018, 2019). Before any conclusions can be drawn from these observations, further quantification should be done with the addition of more cohorts as well as a finer subregion analysis for each region.

Wild-type specific alterations in PNN expression in maternal behavior

In the motor cortex, we identified dynamic alterations in PNN expression between the right and left hemisphere of the SurWT. PNNs are thought to be stable and long-term structures that form after a new experience, solidifying the connections

that have been made between neurons (Miyata and Kitagawa, 2017; Sorg et al., 2016; Ueno et al., 2018), similar to what was seen in the left hemisphere of the SurWT motor cortex. In the right hemisphere, we saw a reduction in PNN expression after behavior, suggesting that the reduction in PNNs is required for efficient pup gathering behavior. PNNs are degraded in the brain by endogenous metallopeptides and hyaluronidases during periods of remodeling (Lorenzo Bozzelli et al., 2018; Lu et al., 2011; Mayer et al., 2005; Miyata and Kitagawa, 2017). The purpose of this reduction could be to allow new connections to be formed between neurons during a period of heightened plasticity, like learned maternal behavior.

Our analysis of the dentate gyrus, visual, caudomedial entorhinal, and insular cortices show that not all regions of the brain have changes in PNN density as a result of learned maternal behavior in the WT. This is not to say that these other regions are not undergoing experience-dependent plasticity changes, as we did not look at other markers of maturing networks like PV, GAD67 or GABA that have been shown to also increase with PNN formation in the cortex specifically (Donato et al., 2013; Gogolla et al., 2014; Hou et al., 2017). Similarly, this shows that not all regions undergo PNN alteration in the *Mecp2^{Het}* mice after behavior, showing that MECP2 deficiency does not result in global dysregulation of PNNs during this experience-dependent event.

PNN expression is increased in SurHet primary somatosensory cortex

We have identified the primary somatosensory cortex as a region of interest that may be contributing to the inefficient pup gathering behavior alongside the auditory cortex. Tactile feedback from pups has been established as one of the primary sensory cues to initiate the onset of maternal behaviors in first-time mothers and in naïve virgin female rodents (Alsina-Llanes et al., 2015; Kenyon et al., 1981; Lonstein and Stern, 1997; Morgan et al., 1992; Stern and Kolunje, 1989). If increased PNNs in this region are restricting plasticity in the Het, as they were in the auditory cortex, this could be a major barrier to the learning of the maternal paradigm. Further study of the primary somatosensory cortex, split into functional subregions, could help us to identify specific tactile sensory cues that are involved in the pup gathering behavior.

Within the Rett Syndrome field, developmental studies in the *Mecp2*-null males have identified morphological deficits in primary somatosensory cortex, such as reduced dendritic complexity and smaller overall area representation (Lee et al., 2017; Moroto et al., 2013). These morphological differences are accompanied by a reduction in cortical signaling (Dani et al., 2005), which correlates with the upregulation of the inhibitory network in MECP2 deficient mice (Chao et al., 2010; He et al., 2014). While this exact phenotype in the *Mecp2*^{Het} somatosensory cortex has not yet been reported, the increase of PNNs in the SurHet could be indicative of a dysregulated inhibitory network, as in the auditory cortex (Krishnan et al., 2017). We suggest that future studies of this region be done in a whole brain, and subregion-specific manner to elucidate a potential other sensory region that is being affected by MECP2 deficiency in the pup gathering behavior.

Circuit-specific alterations in PNN expression

The maternal behavior is a complex multisensory paradigm with well-studied circuitry. We show in our qualitative analysis that many regions involved in the processing of sensory stimuli, like auditory, olfactory, and somatosensory cues, seem to exhibit changes in PNN expression (Tables 1.1-4). These changes may be indicative of experience-dependent plasticity changes that are needed for efficient pup gathering behavior (Tables 1.1-4, column 3) or indicative of dysregulation of experience-dependent plasticity mechanisms in the Het which are disrupting pup gathering behavior (Tables 1.1-4, column 4). As these are well-established circuits, it would be naïve of us to claim that dysregulation of PNN expression in one or two regions are causing the strong behavioral phenotype that we are seeing in the SurHets. We have established that PNNs in the auditory cortex of the SurHet contribute to inefficient pup retrieval (Krishnan et al., 2017) and in this descriptive analysis we have identified other sensory input and integration regions that may be contributing to this inefficient pup gathering behavior as well. While we explored only a few of these regions in more depth, we can always come back to this qualitative analysis and begin PNN quantification on other regions.

References

- Alsina-Llanes, M., Victoria De Brun, and Olazábal, D.E. (2015). Development and expression of maternal behavior in naïve female C57BL/6 mice: Ontogeny of Maternal Behavior in Mice. *Developmental Psychobiology* 57, 189–200.
- Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* 23, 185–188.
- Arkley, K., Grant, R.A., Mitchinson, B., and Prescott, T.J. (2014). Strategy change in vibrissal active sensing during rat locomotion. *Curr. Biol.* 24, 1507–1512.
- Banerjee, S.B., Gutzeit, V.A., Baman, J., Aoued, H.S., Doshi, N.K., Liu, R.C., and Ressler, K.J. (2017). Perineuronal Nets in the Adult Sensory Cortex Are Necessary for Fear Learning. *Neuron* 95, 169-179.e3.
- Beach, F.A., and Jaynes, J. (1956). Studies of Maternal Retrieving in Rats. III. Sensory Cues Involved in the Lactating Female's Response To Her Young. *Behaviour* 10, 104–124.
- Bedogni, F., Cobolli Gigli, C., Pozzi, D., Rossi, R.L., Scaramuzza, L., Rossetti, G., Pagani, M., Kilstrup-Nielsen, C., Matteoli, M., and Landsberger, N. (2016). Defects During *Mecp2* Null Embryonic Cortex Development Precede the Onset of Overt Neurological Symptoms. *Cereb. Cortex* 26, 2517–2529.
- Berretta, S., Pantazopoulos, H., Markota, M., Brown, C., and Batzianouli, E.T. (2015). Losing the sugar coating: Potential impact of perineuronal net abnormalities on interneurons in schizophrenia. *Schizophrenia Research* 167, 18–27.
- Biella, G., and de Curtis, M. (2000). Olfactory Inputs Activate the Medial Entorhinal Cortex Via the Hippocampus. *Journal of Neurophysiology* 83, 1924–1931.
- Brecht, M. (2017). The Body Model Theory of Somatosensory Cortex. *Neuron* 94, 985–992.
- Calamandrei, G., and Keverne, E.B. (1994). Differential expression of Fos protein in the brain of female mice dependent on pup sensory cues and maternal experience. *Behavioral Neuroscience* 108, 113–120.
- Carstens, K.E., Phillips, M.L., Pozzo-Miller, L., Weinberg, R.J., and Dudek, S.M. (2016). Perineuronal Nets Suppress Plasticity of Excitatory Synapses on CA2 Pyramidal Neurons. *The Journal of Neuroscience* 36, 6312–6320.
- Cattaud, V., Bezzina, C., Rey, C.C., Lejards, C., Dahan, L., and Verret, L. (2018). Early disruption of parvalbumin expression and perineuronal nets in the hippocampus of the Tg2576 mouse model of Alzheimer's disease can be rescued by enriched environment. *Neurobiol. Aging* 72, 147–158.

- Chao, H.-T., Chen, H., Samaco, R.C., Xue, M., Chahrour, M., Yoo, J., Neul, J.L., Gong, S., Lu, H.-C., Heintz, N., et al. (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* 468, 263–269.
- Cohen, L., Rothschild, G., and Mizrahi, A. (2011). Multisensory integration of natural odors and sounds in the auditory cortex. *Neuron* 72, 357–369.
- Dani, V.S., Chang, Q., Maffei, A., Turrigiano, G.G., Jaenisch, R., and Nelson, S.B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 102, 12560–12565.
- Donato, F., Rompani, S.B., and Caroni, P. (2013). Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* 504, 272–276.
- Ebert, D.H., Gabel, H.W., Robinson, N.D., Kastan, N.R., Hu, L.S., Cohen, S., Navarro, A.J., Lyst, M.J., Ekiert, R., Bird, A.P., et al. (2013). Activity-dependent phosphorylation of MeCP2 threonine 308 regulates interaction with NCoR. *Nature* 499, 341–345.
- Feldman, D.E., and Brecht, M. (2005). Map plasticity in somatosensory cortex. *Science* 310, 810–815.
- Galindo-Leon, E.E., Lin, F.G., and Liu, R.C. (2009). Inhibitory plasticity in a lateral band improves cortical detection of natural vocalizations. *Neuron* 62, 705–716.
- Gogolla, N., Caroni, P., Lüthi, A., and Herry, C. (2009). Perineuronal nets protect fear memories from erasure. *Science* 325, 1258–1261.
- Gogolla, N., Takesian, A.E., Feng, G., Fagiolini, M., and Hensch, T.K. (2014). Sensory Integration in Mouse Insular Cortex Reflects GABA Circuit Maturation. *Neuron* 83, 894–905.
- Grant, R.A., Mitchinson, B., Fox, C.W., and Prescott, T.J. (2009). Active touch sensing in the rat: anticipatory and regulatory control of whisker movements during surface exploration. *J. Neurophysiol.* 101, 862–874.
- Hartig, W., Brauer, K., and Bruckner, G. (1992). Wisteria floribunda agglutinin-labelled nets surround parvalbumin-containing neurons. *Neuroreport* 3, 869–872.
- He, L.J., Liu, N., Cheng, T.L., Chen, X.J., Li, Y.D., Shu, Y.S., Qiu, Z.L., and Zhang, X.H. (2014). Conditional deletion of Mecp2 in parvalbumin-expressing GABAergic cells results in the absence of critical period plasticity. *Nature Communications* 5, 5036.
- Hou, X., Yoshioka, N., Tsukano, H., Sakai, A., Miyata, S., Watanabe, Y., Yanagawa, Y., Sakimura, K., Takeuchi, K., Kitagawa, H., et al. (2017). Chondroitin Sulfate Is Required for Onset and Offset of Critical Period Plasticity in Visual Cortex. *Scientific Reports* 7.
- Kenyon, P., Cronin, P., and Keeble, S. (1981). Disruption of maternal retrieving by perioral anesthesia. *Physiology & Behavior* 27, 313–321.

- Kenyon, P., Cronin, P., and Keeble, S. (1983). Role of the infraorbital nerve in retrieving behavior in lactating rats. *Behavioral Neuroscience* 97, 255–269.
- Kishi, N., and Macklis, J.D. (2004). MECP2 is progressively expressed in post-migratory neurons and is involved in neuronal maturation rather than cell fate decisions. *Molecular and Cellular Neuroscience* 27, 306–321.
- Kochlamazashvili, G., Henneberger, C., Bukalo, O., Dvoretzkova, E., Senkov, O., Lievens, P.M.-J., Westenbroek, R., Engel, A.K., Catterall, W.A., Rusakov, D.A., et al. (2010). The extracellular matrix molecule hyaluronic acid regulates hippocampal synaptic plasticity by modulating postsynaptic L-type Ca(2+) channels. *Neuron* 67, 116–128.
- Kohl, J., Autry, A.E., and Dulac, C. (2017). The neurobiology of parenting: A neural circuit perspective. *Bioessays* 39, 1–11.
- Krishnan, K., Wang, B.-S., Lu, J., Wang, L., Maffei, A., Cang, J., and Huang, Z.J. (2015). MeCP2 regulates the timing of critical period plasticity that shapes functional connectivity in primary visual cortex. *Proc. Natl. Acad. Sci. U.S.A.* 112, E4782-4791.
- Krishnan, K., Lau, B.Y.B., Ewall, G., Huang, Z.J., and Shea, S.D. (2017). MECP2 regulates cortical plasticity underlying a learned behaviour in adult female mice. *Nature Communications* 8, 14077.
- Lau, B.Y.B., Krishnan, K., Huang, Z.J., and Shea, S.D. (2020). Maternal Experience-Dependent Cortical Plasticity in Mice Is Circuit- and Stimulus-Specific and Requires MECP2. *The Journal of Neuroscience* 40, 1514–1526.
- Layo, D.E., Lau, B.Y.B., and Krishnan, K. (2020). Mapping and Counting High-Intensity Perineuronal Nets in the Somatosensory Cortex v2 (protocols.io.bcf8itrw).
- Lee, L.-J., Tsytsarev, V., and Erzurumlu, R.S. (2017). Structural and functional differences in the barrel cortex of *Mecp2* null mice. *Journal of Comparative Neurology* 525, 3951–3961.
- Lee, S., Kruglikov, I., Huang, Z.J., Fishell, G., and Rudy, B. (2013). A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nature Neuroscience* 16, 1662–1670.
- Lonstein, J.S., and Stern, J.M. (1997). Somatosensory contributions to c-fos activation within the caudal periaqueductal gray of lactating rats: effects of perioral, rooting, and suckling stimuli from pups. *Horm Behav* 32, 155–166.
- Lonstein, J.S., and Stern, J.M. (1999). Effects of unilateral suckling on nursing behavior and c-fos activity in the caudal periaqueductal gray in rats. *Developmental Psychobiology* 35, 264–275.
- Lonstein, J.S., Lévy, F., and Fleming, A.S. (2015). Common and divergent psychobiological mechanisms underlying maternal behaviors in non-human and human mammals. *Hormones and Behavior* 73, 156–185.

- Lorenzo Bozzelli, P., Alaiyed, S., Kim, E., Villapol, S., and Conant, K. (2018). Proteolytic Remodeling of Perineuronal Nets: Effects on Synaptic Plasticity and Neuronal Population Dynamics. *Neural Plasticity* 2018, 5735789.
- Lu, P., Takai, K., Weaver, V.M., and Werb, Z. (2011). Extracellular Matrix Degradation and Remodeling in Development and Disease. *Cold Spring Harbor Perspectives in Biology* 3, a005058–a005058.
- Mayer, J., Hamel, M.G., and Gottschall, P.E. (2005). Evidence for proteolytic cleavage of brevican by the ADAMTSS in the dentate gyrus after excitotoxic lesion of the mouse entorhinal cortex. *BMC Neurosci* 6, 52.
- Miyata, S., and Kitagawa, H. (2017). Formation and remodeling of the brain extracellular matrix in neural plasticity: Roles of chondroitin sulfate and hyaluronan. *Biochimica et Biophysica Acta* 1861, 2420–2434.
- Morgan, H.D., Fleming, A.S., and Stern, J.M. (1992). Somatosensory control of the onset and retention of maternal responsiveness in primiparous Sprague-Dawley rats. *Physiology & Behavior* 51, 549–555.
- Moroto, M., Nishimura, A., Morimoto, M., Isoda, K., Morita, T., Yoshida, M., Morioka, S., Tozawa, T., Hasegawa, T., Chiyonobu, T., et al. (2013). Altered somatosensory barrel cortex refinement in the developing brain of Mecp2-null mice. *Brain Research* 1537, 319–326.
- Murthy, S., Kane, G.A., Katchur, N.J., Lara Mejia, P.S., Obiofuma, G., Buschman, T.J., McEwen, B.S., and Gould, E. (2019). Perineuronal Nets, Inhibitory Interneurons, and Anxiety-Related Ventral Hippocampal Neuronal Oscillations Are Altered by Early Life Adversity. *Biol. Psychiatry* 85, 1011–1020.
- Okabe, S., Nagasawa, M., Kihara, T., Kato, M., Harada, T., Koshida, N., Mogi, K., and Kikusui, T. (2013). Pup odor and ultrasonic vocalizations synergistically stimulate maternal attention in mice. *Behavioral Neuroscience* 127, 432–438.
- Paxinos, G., and Franklin, K.B.J. (2013). Paxino's and Franklin's The Mouse Brain In Stereotaxic Coordinates, 4th Ed (Waltham, MA: Academic Press).
- Pizzo, R., Gurgone, A., Castroflorio, E., Amendola, E., Gross, C., Sassoè-Pognetto, M., and Giustetto, M. (2016). Lack of Cdkl5 Disrupts the Organization of Excitatory and Inhibitory Synapses and Parvalbumin Interneurons in the Primary Visual Cortex. *Front Cell Neurosci* 10, 261.
- Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J.W., and Maffei, L. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. *Science* 298, 1248–1251.
- Reinhard, S.M., Rais, M., Afroz, S., Hanania, Y., Pendi, K., Espinoza, K., Rosenthal, R., Binder, D.K., Ethell, I.M., and Razak, K.A. (2019). Reduced perineuronal net expression in Fmr1 KO mice auditory cortex and amygdala is linked to impaired fear-associated memory. *Neurobiol Learn Mem* 164, 107042.

- Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675.
- Sigal, Y.M., Bae, H., Bogart, L.J., Hensch, T.K., and Zhuang, X. (2019). Structural maturation of cortical perineuronal nets and their perforating synapses revealed by superresolution imaging. *Proc. Natl. Acad. Sci. U.S.A.* 116, 7071–7076.
- Skene, P.J., Illingworth, R.S., Webb, S., Kerr, A.R.W., James, K.D., Turner, D.J., Andrews, R., and Bird, A.P. (2010). Neuronal MeCP2 Is Expressed at Near Histone-Octamer Levels and Globally Alters the Chromatin State. *Molecular Cell* 37, 457–468.
- Sorg, B.A., Berretta, S., Blacktop, J.M., Fawcett, J.W., Kitagawa, H., Kwok, J.C.F., and Miquel, M. (2016). Casting a Wide Net: Role of Perineuronal Nets in Neural Plasticity. *The Journal of Neuroscience* 36, 11459–11468.
- Sreenivasan, V., Karmakar, K., Rijli, F.M., and Petersen, C.C.H. (2015). Parallel pathways from motor and somatosensory cortex for controlling whisker movements in mice. *European Journal of Neuroscience* 41, 354–367.
- Stefanelli, G., Gandaglia, A., Costa, M., Cheema, M.S., Di Marino, D., Barbiero, I., Kilstrup-Nielsen, C., Ausió, J., and Landsberger, N. (2016). Brain phosphorylation of MeCP2 at serine 164 is developmentally regulated and globally alters its chromatin association. *Sci Rep* 6, 28295.
- Stern, J.M. (1990). Multisensory regulation of maternal behavior and masculine sexual behavior: A revised view. *Neuroscience & Biobehavioral Reviews* 14, 183–200.
- Stern, J.M. (1996). Somatosensation and Maternal Care in Norway Rats. In *Advances in the Study of Behavior*, (Elsevier), pp. 243–294.
- Stern, J.M., and Kolunje, J.M. (1989). Perioral anesthesia disrupts maternal behavior during early lactation in Long-Evans rats. *Behav. Neural Biol.* 52, 20–38.
- Stettler, D.D., and Axel, R. (2009). Representations of Odor in the Piriform Cortex. *Neuron* 63, 854–864.
- Thompson, E.H., Lensjø, K.K., Wigestrang, M.B., Malthe-Sørensen, A., Hafting, T., and Fyhn, M. (2018). Removal of perineuronal nets disrupts recall of a remote fear memory. *Proceedings of the National Academy of Sciences* 115, 607–612.
- Ueno, H., Suemitsu, S., Okamoto, M., Matsumoto, Y., and Ishihara, T. (2017). Sensory experience-dependent formation of perineuronal nets and expression of Cat-315 immunoreactive components in the mouse somatosensory cortex. *Neuroscience* 355, 161–174.
- Ueno, H., Takao, K., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Okamoto, M., Aoki, S., and Ishihara, T. (2018). Age-dependent and region-specific alteration of parvalbumin neurons and perineuronal nets in the mouse cerebral cortex. *Neurochemistry International* 112, 59–70.
- Ueno, H., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Matsumoto, Y., Okamoto, M., and Ishihara, T. (2019). Layer-specific expression of extracellular matrix molecules in the mouse somatosensory and piriform cortices. *IBRO Reports* 6, 1–17.

Veinante, P., and Deschênes, M. (2003). Single-cell study of motor cortex projections to the barrel field in rats: Motor Cortex Projections to the Barrel Field. *Journal of Comparative Neurology* 464, 98–103.

Walsh, C.J., Fleming, A.S., Lee, A., and Magnusson, J.E. (1996). The effects of olfactory and somatosensory desensitization on Fos-like immunoreactivity in the brains of pup-exposed postpartum rats. *Behavioral Neuroscience* 110, 134–153.

Welker, W., Sanderson, K.J., and Shambes, G.M. (1984). Patterns of afferent projections to transitional zones in the somatic sensorimotor cerebral cortex of albino rats. *Brain Research* 292, 261–267.

Zhou, Z., Hong, E.J., Cohen, S., Zhao, W., Ho, H.H., Schmidt, L., Chen, W.G., Lin, Y., Savner, E., Griffith, E.C., et al. (2006). Brain-Specific Phosphorylation of MeCP2 Regulates Activity-Dependent Bdnf Transcription, Dendritic Growth, and Spine Maturation. *Neuron* 52, 255–269.

Appendix

Table 1.1. Qualitative PNN expression changes in the amygdala that occur between before and after pup gathering behavior in the left or right hemisphere between conditions.

Within each column, the ($\uparrow$) indicates the PNN expression appeared higher in the Het (1 & 2) or Surrogate (3 & 4) brain, ($\downarrow$) indicates that PNN expression appeared to decrease in the Het (1 & 2) or Surrogate (3 & 4) brain, (*delta symbol*) indicates that changes in PNN expression were seen over the medial-lateral axis, however there were both increases and reductions in PNN expression within the Het (1 & 2) or Surrogate (3 & 4) brain across that axis. Blank squares were regions where no change was noticed. Column 1 and 2 shows genotype differences between the Naïve state, before any new experience, and in the Surrogate state, after the pup gathering experience. Columns 3 and 4 highlight alterations within genotype that are occurring as a result of the behavioral experience. Regions present in a section change across the medial-lateral axis of the brain, so sections were analyzed spanning the whole brain (0.12 mm - 3.72 mm). Qualitative analysis: N = 2 brains per condition.

	Column 1		Column 2		Column 3		Column 4	
	NW / NH		SW / SH		NW / SW		NH / SH	
	Left	Right	Left	Right	Left	Right	Left	Right
Amygdala								
Anterior Amygdaloid Area	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$			$\downarrow$	$\uparrow$
Basolateral Amygdaloid Nucleus			$\uparrow$	$\uparrow$			$\downarrow$	$\uparrow$
Basomedial Amygdaloid Nucleus	$\uparrow$			$\uparrow$			$\downarrow$	
Central Amygdaloid Nucleus	$\uparrow$				$\uparrow$		$\uparrow$	$\uparrow$
Cortex-amygdala transition zone	$\uparrow$							
Medial Amygdaloid Nucleus	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$		$\triangle$	$\triangle$

Table 1.2. Qualitative PNN expression changes in the hippocampus that occur between before and after pup gathering behavior in the left or right hemisphere between conditions.

Within each column, the ($\uparrow$) indicates the PNN expression appeared higher in the Het (1 & 2) or Surrogate (3 & 4) brain, ($\downarrow$) indicates that PNN expression appeared to decrease in the Het (1 & 2) or Surrogate (3 & 4) brain, (*delta symbol*) indicates that changes in PNN expression were seen over the medial-lateral axis, however there were both increases and reductions in PNN expression within the Het (1 & 2) or Surrogate (3 & 4) brain across that axis. Blank squares were regions where no change was noticed. Column 1 and 2 shows genotype differences between the Naïve state, before any new experience, and in the Surrogate state, after the pup gathering experience. Columns 3 and 4 highlight alterations within genotype that are occurring as a result of the behavioral experience. PNN expression was later quantified in the regions highlighted in gray. Regions present in a section change across the medial-lateral axis of the brain, so sections were analyzed spanning the whole brain (0.12 mm - 3.72 mm). Qualitative analysis: N = 2 brains per condition.

	Column 1		Column 2		Column 3		Column 4	
	NW / NH		SW / SH		NW / SW		NH / SH	
	Left	Right	Left	Right	Left	Right	Left	Right
Hippocampus								
CA1	$\uparrow$		$\uparrow$	$\downarrow$				$\uparrow$
CA2	$\uparrow$	$\uparrow$	$\uparrow$	$\triangle$	$\triangle$	$\uparrow$	$\downarrow$	$\downarrow$
CA3				$\uparrow$	$\downarrow$	$\uparrow$		$\uparrow$
Dentate Gyrus			$\uparrow$			$\uparrow$		
Subiculum (dorsal)	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\triangle$	$\triangle$	$\uparrow$	$\uparrow$
Subiculum (para + post)	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$

Table 1.3. Qualitative PNN expression changes in cortical regions that occur between before and after pup gathering behavior in the left or right hemisphere between conditions.

Within each column, the ($\uparrow$) indicates the PNN expression appeared higher in the Het (1 & 2) or Surrogate (3 & 4) brain, ($\downarrow$) indicates that PNN expression appeared to decrease in the Het (1 & 2) or Surrogate (3 & 4) brain, (*delta symbol*) indicates that changes in PNN expression were seen over the medial-lateral axis, however there were both increases and reductions in PNN expression within the Het (1 & 2) or Surrogate (3 & 4) brain across that axis. Blank squares were regions where no change was noticed. Column 1 and 2 shows genotype differences between the Naïve state, before any new experience, and in the Surrogate state, after the pup gathering experience. Columns 3 and 4 highlight alterations within genotype that are occurring as a result of the behavioral experience. PNN expression was later quantified in the regions highlighted in gray. Regions present in a section change across the medial-lateral axis of the brain, so sections were analyzed spanning the whole brain (0.12 mm - 3.72 mm). Qualitative analysis: N = 2 brains per condition.

	Column 1		Column 2		Column 3		Column 4	
	NW / NH		SW / SH		NW / SW		NH / SH	
	Left	Right	Left	Right	Left	Right	Left	Right
Cortex								
Auditory Cortex	$\uparrow$	$\uparrow$		$\uparrow$	$\uparrow$			
Caudomedial Entorhinal Cortex	$\uparrow$	Δ	$\uparrow$	$\uparrow$	$\uparrow$		$\uparrow$	$\uparrow$
Insular Cortices (agranular)	Δ	$\uparrow$	$\uparrow$	$\uparrow$	Δ	Δ	$\uparrow$	$\uparrow$
Insular Cortices (granular)	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$		$\downarrow$		$\uparrow$
Primary Motor Cortex	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	Δ	Δ
Secondary Motor Cortex	$\uparrow$	$\downarrow$	$\uparrow$	$\uparrow$	$\uparrow$		$\uparrow$	$\uparrow$
Parietal Association Cortex		$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$		$\uparrow$	$\uparrow$
Piriform Cortex	Δ	$\uparrow$	$\uparrow$	$\uparrow$		Δ	$\uparrow$	$\uparrow$
Primary Somatosensory Barrel Field	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$	Δ	$\uparrow$
Primary Somatosensory Upper Lip	$\downarrow$	$\uparrow$	$\uparrow$	$\uparrow$	Δ	Δ	$\uparrow$	$\uparrow$
Primary Somatosensory Forelimb	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$	$\uparrow$
Visual Cortex (primary)	$\uparrow$		$\uparrow$	Δ	$\uparrow$	$\downarrow$	$\uparrow$	Δ
Visual Cortex (secondary)		$\uparrow$	$\downarrow$			Δ	$\uparrow$	Δ

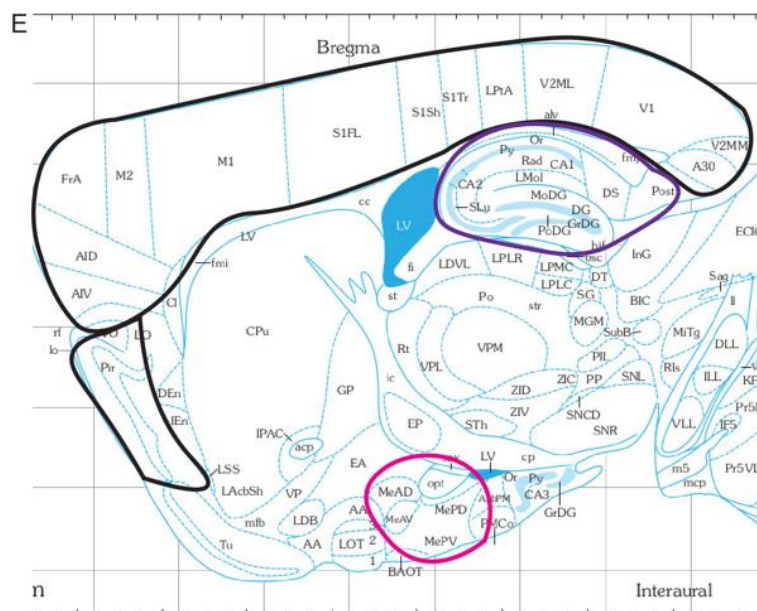
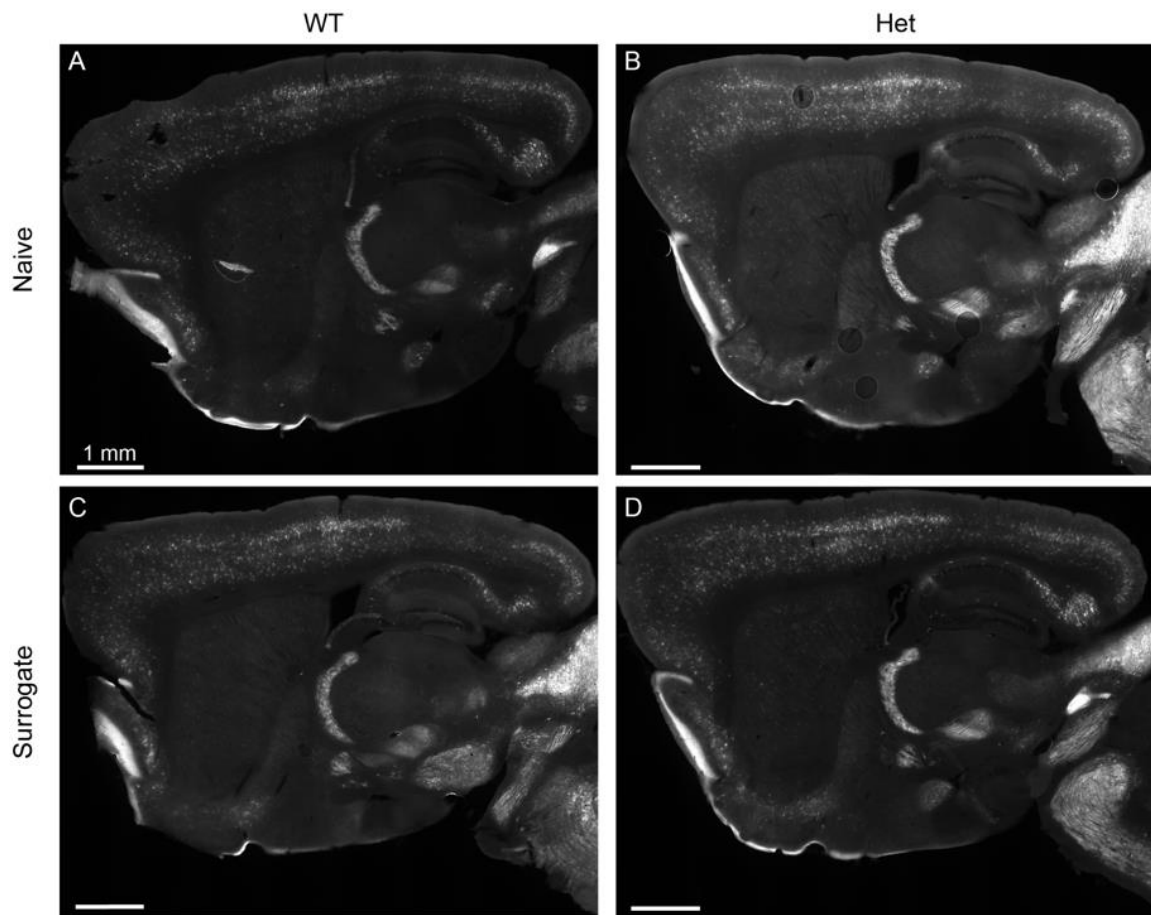
Table 1.4. Qualitative PNN expression changes in other subcortical regions that occur between before and after pup gathering behavior in the left or right hemisphere between conditions.

Within each column, the ($\uparrow$) indicates the PNN expression appeared higher in the Het (1 & 2) or Surrogate (3 & 4) brain, ($\downarrow$) indicates that PNN expression appeared to decrease in the Het (1 & 2) or Surrogate (3 & 4) brain, (*delta symbol*) indicates that changes in PNN expression were seen over the medial-lateral axis, however there were both increases and reductions in PNN expression within the Het (1 & 2) or Surrogate (3 & 4) brain across that axis. Blank squares were regions where no change was noticed. Column 1 and 2 shows genotype differences between the Naïve state, before any new experience, and in the Surrogate state, after the pup gathering experience. Columns 3 and 4 highlight alterations within genotype that are occurring as a result of the behavioral experience. Regions present in a section change across the medial-lateral axis of the brain, so sections were analyzed spanning the whole brain (0.12 mm - 3.72 mm). Qualitative analysis: N = 2 brains per condition.

	Column 1		Column 2		Column 3		Column 4	
	NW / NH		SW / SH		NW / SW		NH / SH	
	Left	Right	Left	Right	Left	Right	Left	Right
Subcortical areas and thalamic nuclei								
Caudate Putamen (striatum)	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$				$\uparrow$
Global Pallidus	$\uparrow$		$\downarrow$					$\uparrow$
Internal capsule	$\uparrow$	Δ		$\uparrow$		$\downarrow$		$\uparrow$
Nucleus of the lateral olfactory tract	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$				
Periaqueductal gray		$\uparrow$			$\downarrow$			
Reticular Nucleus	$\uparrow$	$\uparrow$		$\uparrow$		$\uparrow$		
Substantia Nigra	Δ	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\uparrow$		
Subthalamic nucleus	$\uparrow$	$\uparrow$			$\uparrow$			
Olfactory tubercle				$\uparrow$				$\uparrow$

Figure 1.1. Schema of the descriptive analysis of PNN expression changes between conditions within different regions.

Representative whole section epifluorescent images of map location 1.80 mm (E) from the left hemisphere of NaïveWT (A), NaïveHet (B), SurWT (C), and SurHet (D). Region comparisons were based on the map template (E) from the Paxinos and Franklin The Mouse Brain Atlas (Paxinos and Franklin, 2013). These comparisons were recorded in Table 1.1 as increase/decrease across the brain. E. The regions were isolated from the cortex (outlined in black), hippocampus (outlined in purple), amygdala (outlined in pink) and other subcortical regions (not outlined). The regions that are present in a section change along the medial-lateral axis of the brain, so sections were analyzed spanning the whole brain in both hemispheres (0.12 mm – 3.72 mm). Images for this analysis were taken at 20x and stitched together (n=2 mice per genotype)



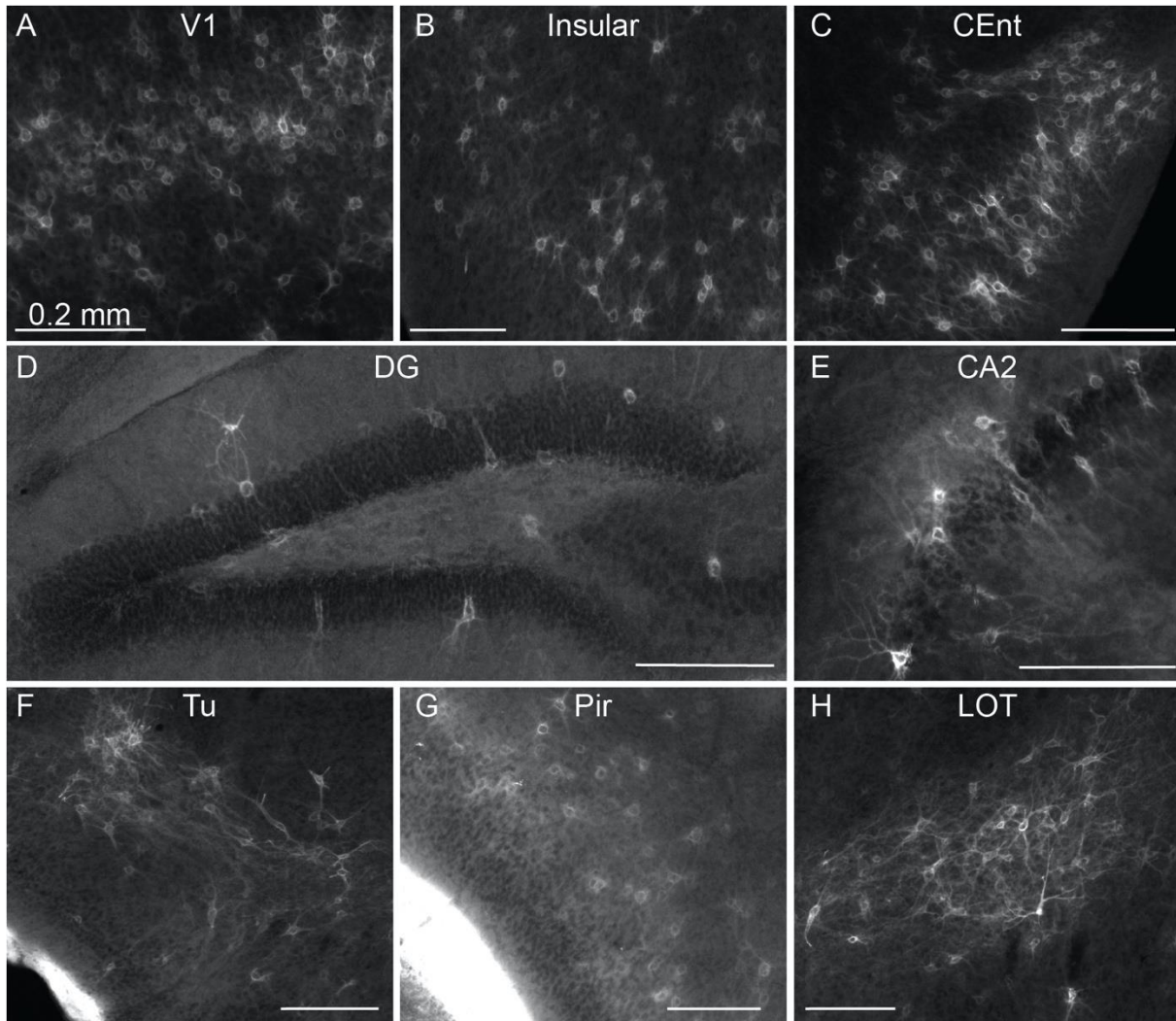


Figure 1.2. PNN structure and density differ between brain regions.

Representative images of PNN expression from cortical (A-C, G), hippocampal (D & E) and subcortical (F & H) regions of the brain. Cortical regions such as the visual and entorhinal cortex (A & C) have more densely packed PNNs than the insular or piriform cortices (B & G). PNNs in the piriform cortex are more diffuse in structure, similar to the dentate gyrus (D) and olfactory tubercle (F). The piriform cortex (G) and CA2 (E) regions have a hazy background signal that persists across conditions and between cohorts. Subcortical regions, like the olfactory tubercle (F) and the nucleus of the lateral olfactory tract (H) show that PNNs surround more of the axons and proximal dendrites than in the cortex. All images were taken at the optimized exposure setting for that region, in a 20x z-series that was then compressed and stitched together.

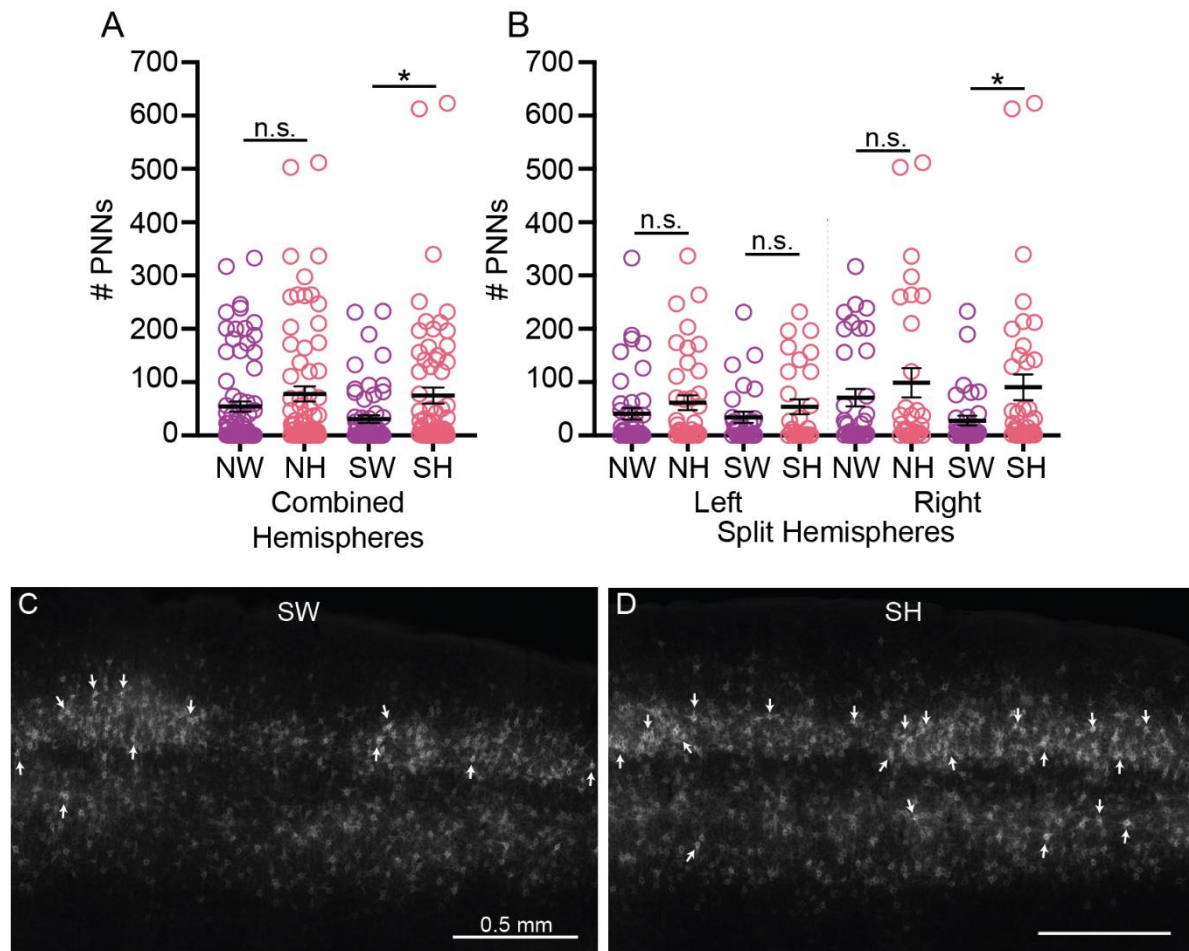
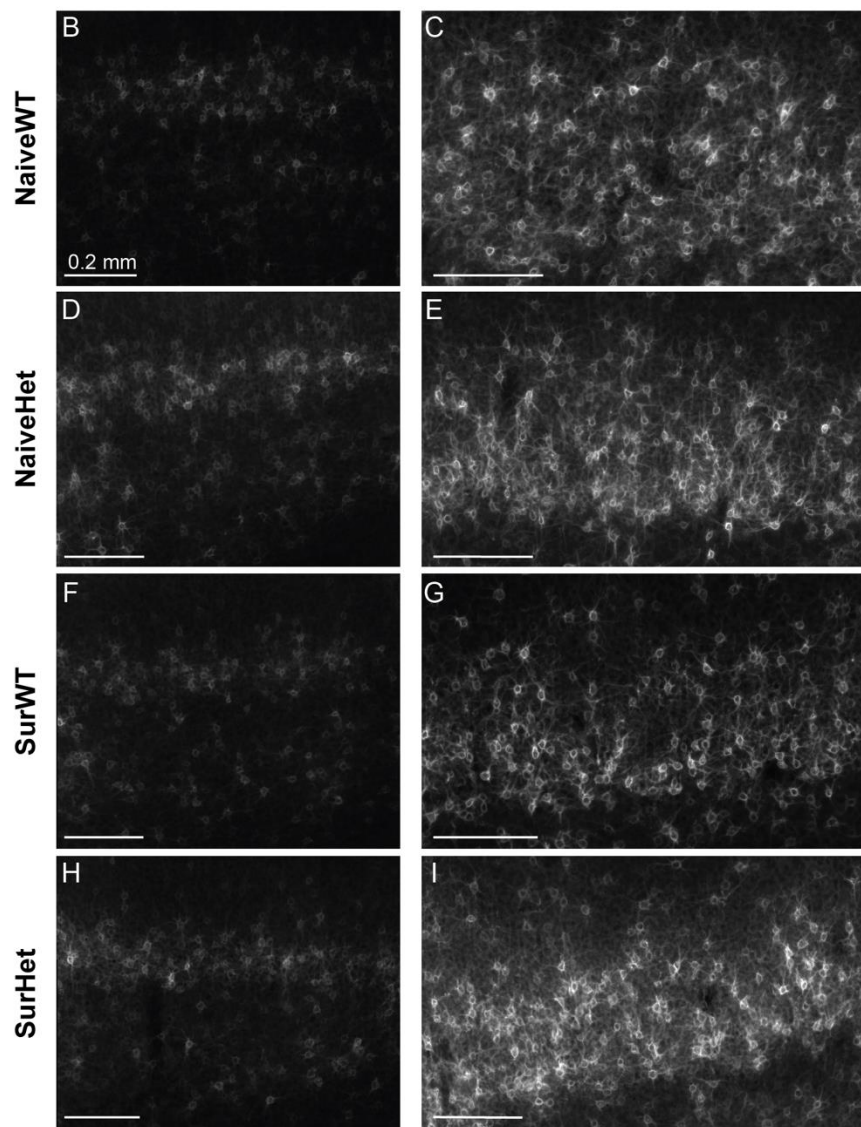
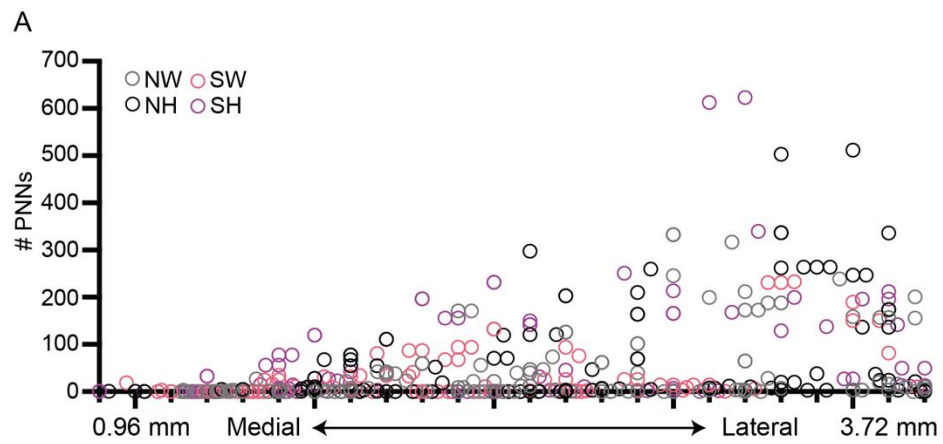


Figure 1.4. Surrogacy increases PNN expression in the Het primary somatosensory cortex when compared to WT.

A-B. Quantification of PNN expression across the primary somatosensory cortex in a whole brain (A) and hemisphere-specific manner (B). Each circle represents an individual brain section. C-D. Representative 20x stitched images of high-intensity PNN expression in the right primary somatosensory cortex of the SW (C) and SH (D). High-intensity PNNs indicated by white arrows. PNN expression was increased in the SH when compared to SW in the combined dataset (A) as well as in the right hemisphere (B). This may be due to a reduction in PNN expression in the SW when compared to NW, however this trend was not statistically significant. WT in purple, Het in peach. *Kruskal-Wallis followed by uncorrected Dunn's test*, * $p < 0.05$, n.s. no significance. Animals: $n = 3$ per genotype; sections: $n =$ Whole brain: NW 77; NH 70; SW 63; SH 68 :: Hemisphere: Left: NW 42; NH 39; SW 28; SH 29 :: Right: NW 35, NH 31, SW 35, SH 39

Figure 1.5. PNN expression increases along the medial-lateral axis of the primary somatosensory cortex, regardless of condition.

A. Quantification of PNN expression across the entire medial-lateral axis of the primary somatosensory cortex. Each dot represents the number of PNNs in one section, the conditions are color coded. B-I. Representative 20x stitched images of high-intensity PNN expression in medial (B, D, F, H) and lateral (C, E, G, I) sections of the primary somatosensory cortex of the NW (B-C), NH (D-E), SW (F-G) and SH (H-I). PNN expression increases in the lateral sections of the primary somatosensory cortex when compared with medial sections, regardless of genotype. PNNs are brighter and more densely packed in the lateral sections when compared to the medial sections, even though all sections were imaged using the same exposure time. Medial sections of the somatosensory cortex contain functional subregions related to tactile input from the trunk and hindlimbs, while the lateral sections process tactile input from the face and whiskers. As this difference is present across conditions, it is possible that PNNs may play a larger role in facial tactile input than that of the rest of the body. Animals: n=3 per genotype; sections: n= NW 77; NH 70; SW 63; SH 68



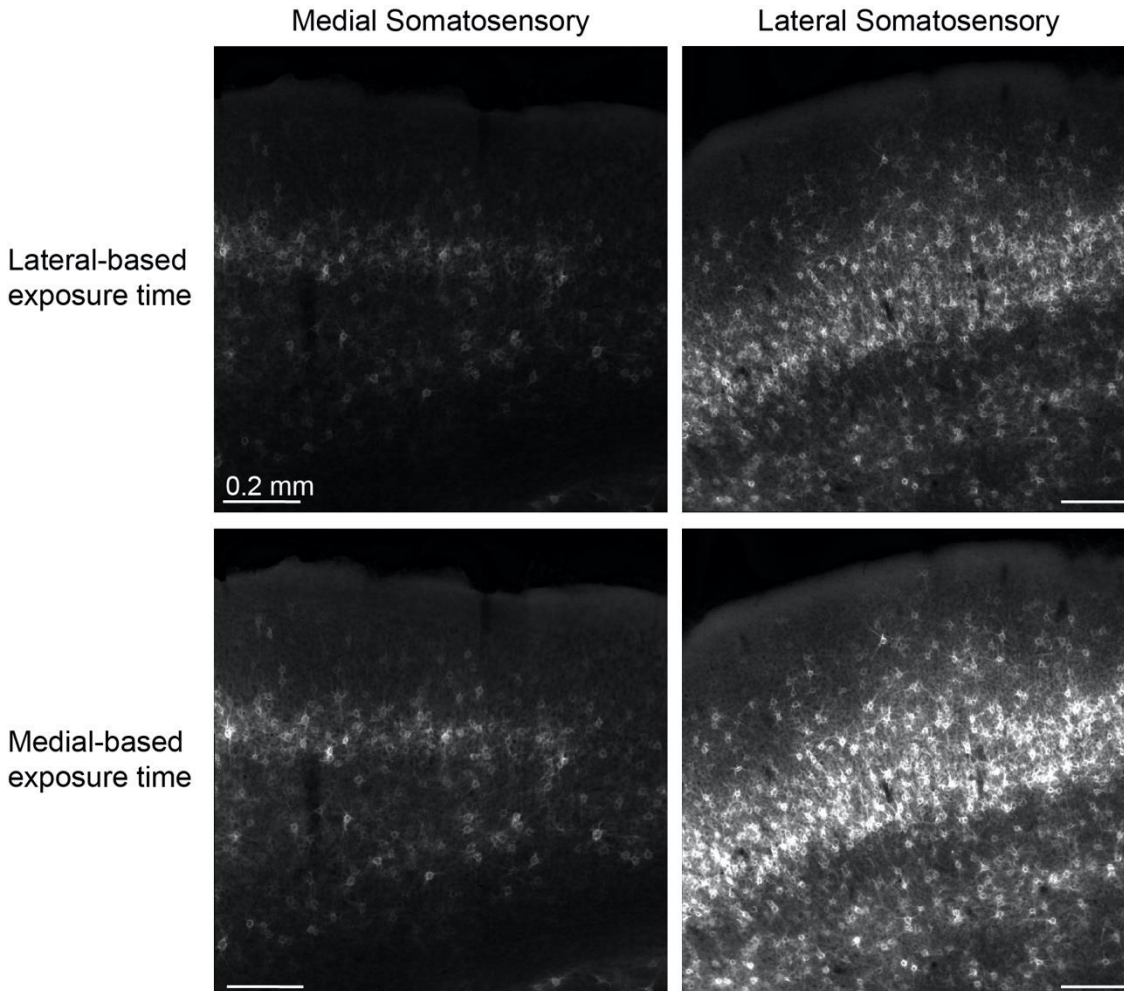
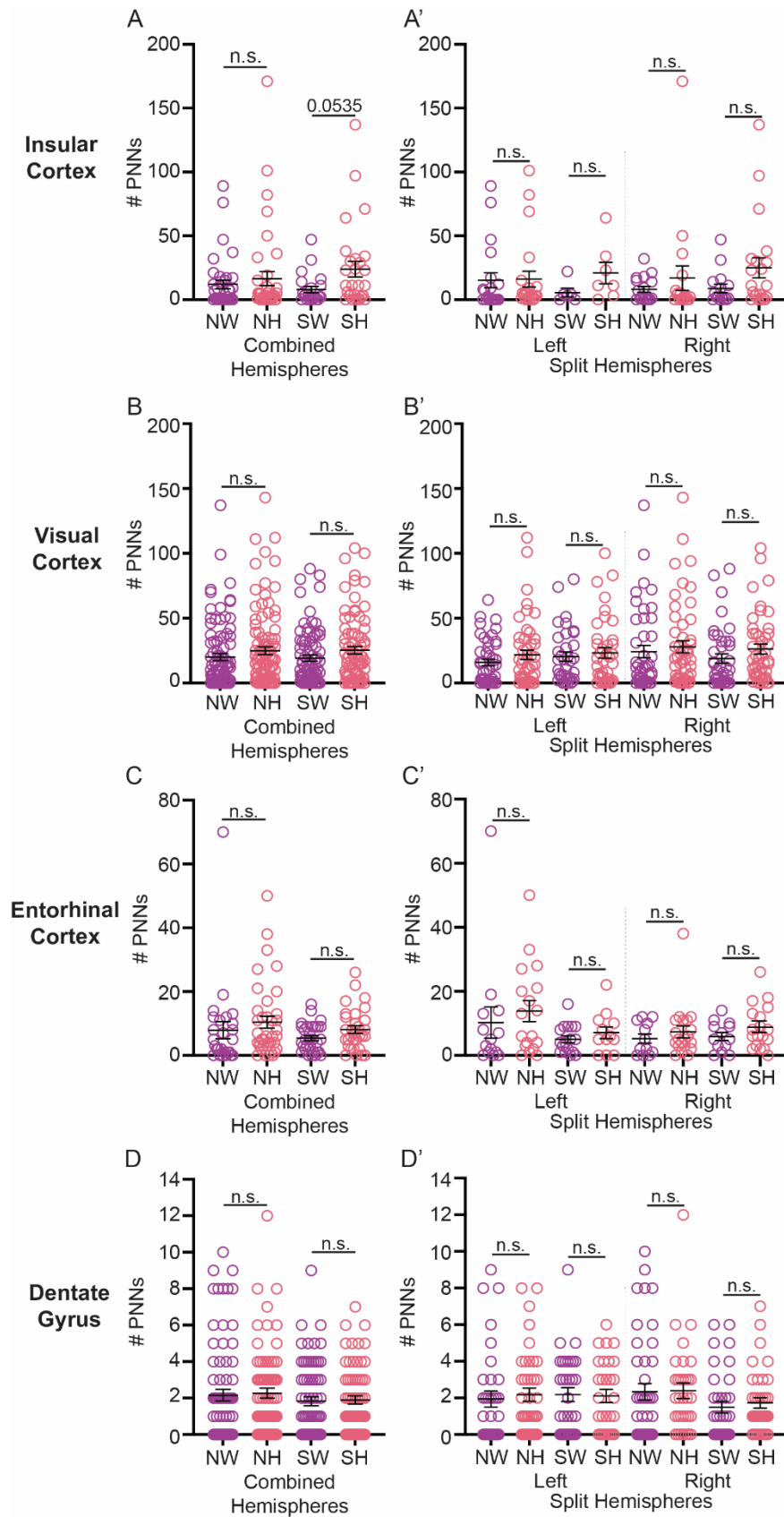


Figure 1.6. Imaging settings across the medial-lateral axis in the primary somatosensory cortex.

Exposure times for imaging of a full cohort are based on the average exposure time within the left hemisphere of the SH, as this condition typically has the brightest PNNs, and we want to limit overexposure in order to quantify the PNNs in the region. Here are representative medial (column 1) and lateral (column 2) sections of the SH primary somatosensory cortex, which were imaged at the lateral section-based exposure time (row 1) and medial-based exposure time (row 2). At the lateral-based exposure time, the medial sections contain no high-intensity PNNs, and the image looks under exposed. At the medial-based exposure time, the medial sections now contain high-intensity PNNs, though the PNNs are not as high in quantity or densely packed as the lateral sections. At the medial-based exposure time, the lateral section is very overexposed making high-intensity PNN quantification difficult.

Figure 1.7. Alterations in PNN expression do not occur globally within the brain during pup retrieval in the WT or Het.

A-D. Quantification of PNN expression within the insular cortex (A), visual cortex (B), entorhinal cortex (C), and dentate gyrus (D) between conditions. Each circle represents an individual brain section. None of the regions exhibited any changes in PNN expression between conditions as in the combined or split hemisphere measurements. However, some interesting trends in the data were noted. A-A'. The insular cortex did show, in the combined data, that the SW had less PNNs than the SH. A trend that was common in both hemispheres. However, the number of sections for this region were low and this trend could be driven by that factor. C-C'. A trend seen in the combined and left hemisphere analysis of the entorhinal cortex showed a reduction in PNNs after behavior in both genotypes. WT in purple, Het in peach. *Kruskal-Wallis followed by uncorrected Dunn's test*, n.s. no significance. Animals: n=3 per genotype; sections: A & A'. Whole brain: NW 37; NH 40 ; SW 22; SH 28 :: Hemisphere: Left: NW 20; NH 22; SW 6; SH 7 :: Right: NW 17, NH 18, SW 16, SH 21. B & B'. Whole brain: NW 89; NH 98 ; SW 80; SH 80 :: Hemisphere: Left: NW 45; NH 48; SW 35; SH 39 :: Right: NW 44, NH 50, SW 40, SH 45. C & C'. Whole brain: NW 26; NH 38 ; SW 30; SH 30 :: Hemisphere: Left: NW 14; NH 18; SW 17; SH 12 :: Right: NW 12, NH 20, SW 13, SH 18. D & D'. Whole brain: NW 76; NH 73 ; SW 68; SH 66 :: Hemisphere: Left: NW 34; NH 40; SW 33; SH 29 :: Right: NW 42, NH 33, SW 35, SH 37



CHAPTER II
LATERALIZED EXPRESSION OF CORTICAL PERINEURONAL NETS
DURING MATERNAL EXPERIENCE IS DEPENDENT ON MECP2

Text and figures taken from the following:

Lau BYB, Layo DE, Emery E, Everett M, Kumar A, Stevenson P, Reynolds KG, Cherosky A, Bowyer SA, Roth S, Fisher DG, McCord RP, Krishnan K. Lateralized expression of cortical perineuronal nets during maternal experience is dependent on MECP2. *eNeuro* 24 April 2020, 7 (3); DOI: 10.1523/ ENEURO.0500-19.2020

Author contributions: BYBL, DEL and KK supervised the project, designed experiments and analyzed data. KK, BYBL, DEL, ME and DGF performed the behavior experiments. BYBL, DEL, ME and BE performed sectioning and immunostaining. DEL imaged sections. BYBL, DEL, BE, KR, PS mapped sections to the reference atlas. BYBL, DEL, BE, ME, AK, PS, KR, AC, SHB and SR contributed significantly to manual counting of PNN structures. RPM performed and interpreted PCA analysis. DGF provided insightful and detailed comments on the manuscript. BYBL, DEL, RPM and KK wrote and edited the manuscript.

Abstract

Cortical neuronal circuits along the sensorimotor pathways are shaped by experience during critical periods of heightened plasticity in early postnatal development. After closure of critical periods, measured histologically by the formation and maintenance of extracellular matrix structures called perineuronal nets (PNNs), the adult mouse brain exhibits restricted plasticity and maturity. Mature PNNs are typically considered to be stable structures that restrict synaptic plasticity on cortical parvalbumin+ GABAergic neurons. Changes in environment (i.e. novel behavioral training) or social contexts (i.e. motherhood) are known to elicit synaptic plasticity in relevant neural circuitry. However, little is known about concomitant changes in the PNNs surrounding the cortical parvalbumin+ GABAergic neurons. Here, we show novel changes in PNN density in the primary somatosensory cortex (SS1) of adult female mice after maternal experience (called Surrogate, Sur), using systematic microscopy analysis of a whole brain region. On average, PNNs were increased in the right barrel field and decreased in the left forelimb regions. Individual mice had left hemisphere dominance in PNN density. Using adult female mice deficient in methyl-CpG-binding protein 2 (MECP2), an epigenetic regulator involved in regulating experience-dependent plasticity, we found that MECP2 is critical for this precise and dynamic expression of PNN. Adult naïve *Mecp2*-heterozygous females (Het) had increased PNN density in specific

subregions in both hemispheres before maternal experience, compared to wildtype (WT) littermate controls. The laterality in PNN expression seen in naïve Het was lost after maternal experience in Surrogate Het mice, suggesting possible intact mechanisms for plasticity. Together, our results identify subregion and hemisphere-specific alterations in PNN expression in adult females, suggesting extracellular matrix plasticity as a possible neurobiological mechanism for adult behaviors in rodents.

Introduction

Perineuronal nets (PNNs) are specialized extracellular matrix structures that can act as physical barriers or modulators of plasticity, restrict axon regeneration, and form molecular brakes that actively control synaptic maturation and function of cortical parvalbumin+ (PV+) GABAergic interneurons that drive gamma oscillations (Bartos et al., 2002; Begum and Sng, 2017; Bernard and Prochiantz, 2016; Carstens et al., 2016; Deepa et al., 2002; Dityatev et al., 2007; Donato et al., 2013; Durand et al., 2012; Frischknecht et al., 2009; Gundelfinger et al., 2010; Hartig et al., 1992; Hou et al., 2017; Kalemaki et al., 2018; Kosaka and Heizmann, 1989; Krishnan et al., 2015, 2017; Nakagawa et al., 1986; Orlando et al., 2012; Pizzorusso et al., 2002, 2006; Sigal et al., 2019; Sugiyama et al., 2009; Suttkus et al., 2012; Ueno et al., 2018; Vo et al., 2013; de Winter et al., 2016; Ye and Miao, 2013). In rodents, mature PNNs in the adult cortex are thought to be stable structures, inhibitory to plasticity, and perhaps play roles in long term memory such as “engrams” (Carstens et al., 2016; Gogolla et al., 2009; Thompson et al., 2018). However, most of these observations are based on postnatal cortical development (when typical connections in neural circuitry are still forming) and models for neurobiological disorders (where neural circuitry development and function have gone awry). Currently, it is unclear if changes in adult PNN expression occur under normal conditions and behavioral contexts.

PNNs are composed of chondroitin sulfate proteoglycans, hyaluronan glycosaminoglycan chains, link proteins and tenascin-R (Bignami et al., 1992; Carulli et al., 2007; Kwok et al., 2010; Miyata and Kitagawa, 2017). Wisteria floribunda agglutinin (WFA) is commonly used as a marker for PNNs in the cortex and other brain

regions (Brückner et al., 1996; Hartig et al., 1992). WFA specifically binds to N-acetyl galactosamine found on most chondroitin sulfate side chains of chondroitin sulfate proteoglycans. In rodents, WFA-labeled PNNs are localized predominantly around soma and proximal dendrites of parvalbumin+ (PV+) GABAergic interneurons of the mature cortex. They interdigitate with synaptic contacts on cortical PV+ GABAergic neurons and regulate experience-dependent synaptic plasticity in the cortex, hippocampus, and amygdala (Cattaui et al., 2018; Gogolla et al., 2009; Krishnan et al., 2015, 2017; Miyata et al., 2012; Murthy et al., 2019; Sigal et al., 2019).

In the human brain, decreased numbers of PNNs are associated with pathological conditions such as decreased memory and motor agility (Brückner et al., 2008; Cabungcal et al., 2013; Enwright et al., 2016; Morawski et al., 2004; Pantazopoulos et al., 2015). We have previously shown that precocious or atypical expression of PNNs caused sensory processing deficits in developing male or adult female mouse models for Rett Syndrome (Krishnan et al., 2015, 2017). Rett Syndrome is a neuropsychiatric disorder predominantly caused by mutations in the X-linked gene, methyl CpG-binding protein 2 (MECP2) (Amir et al., 1999). MECP2 regulates neuronal chromatin architecture and gene transcription in response to neural activity and experience during postnatal life (Becker et al., 2016; Chahrour et al., 2008; Ebert et al., 2013; Skene et al., 2010; Zhou et al., 2006). The known cellular function of MECP2 and the characteristic timing of disease progression led us to hypothesize that MECP2 regulates experience-dependent plasticity in specific neural circuits during windows of enhanced sensory and social experience throughout life; disruptions in timing of these plasticity mechanisms results in atypical responses in behavior. We previously tested this hypothesis using a pup retrieval task in the alloparental care paradigm (Krishnan, Lau et al., 2017).

Parenting is an ethologically relevant social behavior consisting of stereotypic components involving the care and nourishment of young. First-time dams seek and gather wandering/scattered pups back to the nest (pup retrieval), an essential aspect of maternal care. Pup retrieval involves processing of primary sensory cues (auditory, tactile, olfactory) to direct efficient searching and gathering of pups with goal-directed

movements back to the nest (Beach and Jaynes, 1956; Lonstein et al., 2015; Stern, 1996). Virgin female mice (naïve) with no previous maternal experience can execute efficient pup retrieval after co-housing ('surrogates' or 'Sur') with a first-time mother and her pups (Cohen et al., 2011a). This assay allows for interrogation of adult experience-dependent plasticity mechanisms as well as likely non-hormonal, epigenetic mechanisms involving the sensory and motor neural circuits (Stolzenberg and Champagne, 2016). The role of the auditory cortex in pup retrieval and maternal experience is a topic of investigation in many labs (Cohen et al., 2011a; Galindo-Leon et al., 2009; Marlin et al., 2015; Stiebler et al., 1997). They continue to contribute to the understanding of how relevant sensory cues are processed in the maternal brain.

Previously, we found that atypical increase in PNN expression in the auditory cortex of *Mecp2*-heterozygous females caused inefficient pup retrieval (Krishnan, Lau et al., 2017). However, we did not find discernable changes in PNN density in the wild type auditory cortex after successful completion of the pup retrieval task. As PNNs are considered barriers to plasticity, we anticipated reduction in PNN expression in wild type that could facilitate efficient retrieval. There are no known reports of reduction in PNN expression in normal adult wild type brains. Here, we seek to answer if mature PNNs are maintained as stable structures or undergo dynamic expression changes in the primary somatosensory cortex (SS1) of adult female mice after maternal experience. We focused on the SS1 due to its known roles in tactile sensation, which is also important for efficient pup retrieval (Brecht, 2007; Feldman and Brecht, 2005; Kenyon et al., 1981; Morgan et al., 1992). By using WFA as a marker and whole-brain analysis of SS1, we find that mature PNNs in adult SS1 1) are differentially expressed in a hemisphere- and subregion-specific manner, 2) show dynamic expression changes after maternal experience, and 3) are influenced by MECP2, a DNA methylation reader/epigenetic regulator of chromatin and gene expression.

Materials and Methods

Animals

All experiments were performed in adult female mice (10-12 weeks old) that were maintained on a 12hr light-dark cycle (lights on 07:00 h) and received food ad libitum. Genotypes used were CBA/CaJ, *Mecp2^{Het}* (C57BL/6 background; B6.129P2(C)-*Mecp2^{tm1.1Bird}/J*) and *Mecp2^{WT}*-siblings (Guy et al., 2001). All procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and approved by the University of Tennessee-Knoxville Institutional Animal Care and Use Committee.

Pup retrieval behavior

Pup retrieval behavior was performed as previously described (Krishnan, Lau et al., 2017) (Figure 2.1 A). Briefly, we housed two virgin female mice (one *Mecp2^{WT}* and one *Mecp2^{Het}* termed 'naïve', NW and NH respectively) with a first-time pregnant CBA/CaJ female beginning 1-5 days before birth. Upon cohousing, the two naïve mice are now termed 'surrogates' (SW for surrogate *Mecp2^{WT}* and SH for surrogate *Mecp2^{Het}*). Pup retrieval behavior started on the day the pups were born (postnatal day 0; D0) as follows for each adult surrogate mouse: 1) (habituation phase) one adult mouse was habituated with 3-5 pups in the home cage for 5 minutes, 2) (isolation phase) pups were removed from the cage for 2 minutes, and 3) (retrieval phase) pups were returned to home cage, one placed at each corner and at the center (the nest was left empty if there were fewer than 5 pups). Each adult female had maximum of 10 minutes to gather the pups to the original nest. After testing, all animals and pups were returned to the home cage. The same procedure was performed again daily to D5. All behaviors were performed in the dark, during the light cycle (between 9:00 AM and 6:00 PM) and were video recorded.

Immunohistochemistry

Immediately after the behavioral trial on D5, surrogate mice as well as a set of corresponding naïve *Mecp2^{WT}* and *Mecp2^{Het}* mice were perfused with 4% paraformaldehyde/PBS, and brains were extracted and post-fixed overnight at 4°C.

Brains were then treated with 30% sucrose/PBS overnight at room temperature (RT) and sectioned in sagittal orientation using a freezing microtome at 70µm. Free-floating brain sections were immunostained at RT as previously described in Krishnan, Lau et al., 2017, with a few modifications. Briefly, sections were blocked in 10% normal goat serum and 0.5% Triton-X for 3 hours, then incubated with biotin-conjugated Wisteria Floribunda Agglutinin Lectin (labels PNNs; 1:500; Sigma-Aldrich) and rabbit MECP2 primary antibody (1:1000; Cell Signaling) overnight at RT in a 5% normal goat serum/0.25% Triton-X solution. Then, sections were incubated for 4 hours with AlexaFluor-488 and Texas-Red secondary antibodies (1:1000; Invitrogen) in a 5% normal goat serum/0.25% Triton-X solution. Finally, sections were counterstained with the nuclear marker, DAPI (1:1000) for 5 minutes, and mounted in Fluoromount-G (Southern Biotech).

Image Acquisition and analysis

To analyze PNNs, 10X single-plane PNN images of the entire primary somatosensory cortex from each brain slice were acquired on a motorized stage, epifluorescent microscope (Keyence BZ-X710; Keyence Corp., NJ, U.S.A.) and stitched using BZ-X Analyzer (Keyence) (Figure 2.1 B). Our initial observation of PNN intensity showed that SH had the most intense fluorescent signal in SS1. Thus, imaging settings were established based on SH within each cohort of mice, in order to minimize over-exposure. The light exposure time for fluorescent signal acquisition was identified by finding the exposure time where a saturated pixel first appears within frame, then decreasing the exposure time by 1 unit, according to software specifications. This exposure time determination method was applied to all brain sections of SH in each cohort and the mean exposure time was calculated and used for final image acquisition within each cohort.

For PNN image analysis, each stitched image was opened in ImageJ (Schneider et al., 2012). Then, SS1 and somatosensory subregion areas were 1) mapped by overlaying templates from Paxinos and Franklin's "The Mouse Brain, 4th edition" (Paxinos and Franklin, 2013), 2) outlined and 3) measured using the functions in ImageJ. To count high-intensity mature PNNs, the 'Contrast' setting from the

browser was set to the far right to threshold weaker signals. The remaining signals were manually quantified under the classification that a 'mature' PNN is at least 80% of its original shape (before contrast adjustment). Detailed protocol for mapping and counting procedures are now available at [protocols.io.dx.doi.org/10.17504/protocols.io.bcf8itrw](https://doi.org/10.17504/protocols.io.bcf8itrw) (Layo et al., 2020). All statistical analyses and graphs were generated using GraphPad Prism.

For MECP2 expression analysis, a portion of SS1 region was imaged using 20X objective and Keyence microscope, with tiled and stacks of 11 images, which were then collapsed and stitched using the Keyence stitch function. Imaging settings were based on left hemisphere of one brain and applied to the right hemisphere of the same brain. Measurement of MECP2 intensity was performed using ImageJ as follows: 1) for each tiled projection image, an ROI box of 1.5 mm (rostral-caudal) x 0.5 mm (dorsal-ventral) to select a large portion of SS1, which covers layers 2/3-5 where most of the PNNs are localized, 2) individual intensity values were acquired and saved by selecting Analyze, Tools and Save XY Coordinates, 3) exported Excel values were sorted and percentage cumulative frequency distribution were calculated using Microsoft Excel and GraphPad Prism (version 8).

Principal Components Analysis (PCA) on PNN density

We used PNN densities (counts per area) for the entire SS1 across all individuals in the five cohorts. If multiple sections per map number were present, values were averaged across sections to give a single density. In the first PCA analysis, to determine whether the PNN patterns segregated primarily by cohort or (genotype and experience), we kept the data for each individual animal separate and averaged PNN densities across every set of 2 adjacent map regions. Because the scale of raw numbers varied so widely between individual animals, we next median normalized PNN densities within each animal. To do this, we calculate the median PNN density across all map numbers for each individual. Then, each individual PNN density for each map number of that individual was divided by ('normalized by') this median. Finally, these median normalized densities were averaged across all individuals in the same condition. This method of normalization accounts for the variability in the median

across cohorts within the same condition. Some regions for some individuals had no data, and, because PCA does not tolerate missing data, we imputed these missing values by using the average median normalized values for that region from animals in other cohorts in the same condition. By running PCA in R, we obtained a weight for each map number showing the highest variance patterns in PNN densities across the individual animals. We then calculated the projection of each animal onto principal components 1 and 2. K-means clustering was used to calculate the two most evident clusters in this PCA space.

In the second PCA analysis, to determine the major PNN density patterns that distinguish genotypes and experience conditions, we left data for each map number separate, but averaged the median normalized PNN densities across the 5 cohorts for each condition. We then performed PCA to determine the major patterns that distinguish these conditions.

Results

PNN density changes across somatosensory cortical maps

As somatosensation during pup retrieval primarily involves facial/snout areas in surrogates (non-lactating adult females) (Lonstein and Stern, 1997; Morgan et al., 1992; Stern, 1996), we present data collected from the somatosensory cortical regions involved in processing tactile stimuli in the alloparental care paradigm (Figure 2.1 A). According to Paxinos and Franklin's atlas (4th edition), there are eight different anatomical somatosensory cortical subregions (S1) (S1 barrel field 'S1BF', S1 dysgranular zone 'S1DZ', S1 fore limb 'S1FL', S1 jaw 'S1J', S1 upper lip 'S1ULp', S1 trunk 'S1Tr', S1 hind limb 'S1HL' and undefined somatosensory cortex 'S1'), here collectively called primary somatosensory cortex (SS1) (Paxinos and Franklin, 2013). To determine how PNN expression changes across the different SS1 subregions, we took a systematic approach covering the whole SS1, rather than the standard approach of analyzing "representative sample sections" (Figure 2.1 B). We analyzed 40-60 sagittal brain sections (at 70 μ m each, both left and right hemispheres) per animal, in five biological replicates across four conditions [naïve WT (NW), naïve Het (NH), surrogate WT (SW), surrogate Het (SH)] (Figure 2.1 A), as qualitative regional

differences in PNN density were observed in pilot studies in our lab. The SS1 subregions are represented by map numbers 120-132, corresponding to lateral coordinates 2.28 mm (medial region) to 3.72 mm (lateral region), which encompass ~1.5 mm of one mouse brain hemisphere.

PNN expression is predominantly found in deeper layers IV, V and VI in the adult SS1 in both NW (Figure 2.2 A) and SW mice (Figure 2.2 B-C). Across the five cohorts, PNN density (as measured by high-intensity PNN counts over area) was not significantly different between NW and SW across the whole SS1 (Figure 2.2 D). However, we noticed a wide range of distribution of PNN density within individual animals across the cohorts (Figure 2.2 D). In order to determine the source of such variability, we parsed the data according to hemispheres (Figure 2.2 E), across the lateral-medial axis (Figure 2.3) and by subregions (Figure 2.4). No significant difference was observed in PNN density between the left and right hemispheres of NW (Figure 2.2 E); however, the right hemisphere of SW (SW-R) had higher PNN density than the left hemisphere (SW-L) (Figure 2.2 B,C,E), suggesting that higher PNN density in the right hemisphere of SW SS1 might consolidate new tactile information related to the pups and/or the mother.

PNN density is PNN expression counts over area. Overall, SS1 area of the SW is significantly increased, compared to NW (Figure 2.2 F); however, that increase was not specific to a particular hemisphere within experimental condition (NW-L vs. NW-R; SW-L vs. SW-R) (Figure 2.2 G). There is a small but significant area increase in left hemisphere in SW, compared to left hemisphere of NW (Figure 2.2 G). Thus, the increased PNN density in the right hemisphere of SW is mainly due to the PNN expression, and not due to the change in area.

Next, we plotted PNN density in individual sections across the lateral-medial axis (Figure 2.3). We observed the individual variability in sections across map numbers in both NW and SW (Figure 2.3 A). In line with Figure 2.2 D, we did not see any significant differences between NW and SW in the medial-lateral axis (light blue lines across multiple map number subdivisions). However, PNN density is significantly higher in the two most lateral sections compared to the two most medial sections within

conditions (grey p value for NW, black p value for SW). Furthermore, NW had similar PNN density between left (grey) and right (black) hemispheres across the medial-lateral axis (Figure 2.3 B, light blue lines), with the more lateral regions of right NW having slightly but significantly higher PNN density compared to its medial regions (black p value). SW showed many sections across the medial-lateral axis with higher PNN density in the right hemisphere (black) than the left hemisphere (grey), particularly in the most medial sections (Figure 2.3 C, blue line). Within hemisphere, lateral regions of SW-L had significantly higher PNN density than its medial regions (grey p value), while there was no difference between these regions in SW-R (black p value). These findings indicate differential PNN expression in a position-specific, hemisphere-specific manner in the SS1 in both NW and SW.

Changes in PNN density are SS1 subregion-specific

Next, we examined if specific subregions of SS1 were particularly plastic for PNN density between NW and SW. We observed no significant differences in PNN density in subregions S1BF, S1DZ and S1FL when aggregating both hemispheres (Figure 2.4 A-C). For S1BF, a region well-studied for whisker activity that contributes to tactile sensation, PNN density increased significantly and specifically in the right hemisphere for SW, compared to NW (Figure 2.4 A'). This result suggests that increased PNN density in the right hemisphere of S1BF could be a potential site for consolidation of tactile sensory information relevant for executing efficient pup retrieval. Contrary to the pattern in S1BF, S1DZ and S1FL regions showed increased PNN density in the right hemisphere of SW, compared to its left hemisphere (Figure 2.4 B', 2.4 C'). S1DZ has been implicated in proprioceptive functions, such as the movement of joints and stretch of muscle receptors (Chapin and Lin, 1984; Lee and Kim, 2012; Shin Yim et al., 2017; Welker et al., 1984); while S1FL is the sensory representation of the forepaw. Currently, the roles of these regions and PNN contribution in maternal behavior is unclear. Other brain regions (S1ULp, S1J and S1) showed no changes after surrogacy, which further highlights S1BF as a potential site for learning consolidation (Table 2.1). In analyzing left hemisphere-specific data, there was a significant decrease in PNN density in S1FL of SW compared to NW (Figure 2.4 C';

Table 2.1, columns 5, 7). This is the first report, to our knowledge, of decreases in PNN density in a social behavior context in adult brains. Together, these results suggest that PNN density changes in adult females, in a hemisphere- and subregion-specific manner that is conducive for experience-dependent plasticity.

Appropriate PNN expression in SS1 is dependent on MECP2

MECP2 is thought to regulate experience-dependent plasticity mechanisms in an epigenetic manner, in early postnatal development and in adulthood (Cohen et al., 2011b; Dani et al., 2005; Durand et al., 2012; Gabel et al., 2015; Guy et al., 2001; Krishnan et al., 2015, 2017; Lagger et al., 2017; Morello et al., 2018; Muotri et al., 2010; Noutel et al., 2011; Picard and Fagiolini, 2019). We previously tested this hypothesis, using an alloparental care paradigm, and found that *Mecp2*-heterozygous (Het) adult females were inefficient at pup retrieval (Krishnan, Lau et al., 2017). In this study, we identified atypical and transient increases in PNN density in the auditory cortex of surrogate Het (SH), leading to altered responses of PV+ neurons to auditory cues in SH (Lau et al., 2020). Here, we sought to determine if SS1 of SH exhibited similar alterations in PNN density in subregion-specific ways. Comparing naïve Het (NH) to SH, we noticed no significant differences in PNN density in whole SS1 (Figure 2.5 A, similar to WT in Figure 2.2 D), or within hemispheres of SS1 (Figure 2.5 B, unlike WT in Figure 2.2 E). There were no significant changes in SS1 area between NH and SH (Figure 2.5 C; unlike SW in Figure 2.2 F). However, an increased SS1 area in left versus right hemisphere of SH was noted (Figure 2.5 D).

For PNN density analysis across medial-lateral axis (Figure 2.6), PNN density was similar between NH and SH along this axis (Figure 2.6 A, light blue lines), with significantly more PNNs in the lateral regions than the medial regions within conditions (grey p value for NH, black p value for SH). Within NH, there was no difference between left and right hemispheres along medial-lateral axis (Figure 2.6 B; light blue lines). Again, lateral regions expressed significantly more PNNs than the medial regions within each hemisphere (grey p value for NH-L, black p value for NH-R). After surrogacy, SH exhibited significantly more dynamic changes in PNN density across medial-lateral axis between left and right hemisphere (Figure 2.6 C, light blue lines).

Moreover, while PNN densities in lateral and medial regions were not significantly different in SH-R (black p value), lateral regions of SH-L had significantly more PNNs than the medial regions (grey p value).

When we compared PNN density between genotypes, WT and Het (Table 2.1, Figure 2.7), we noticed significant hemisphere-specific and subregion-specific differences, with the maintenance of medial-lateral axis. NH had increased PNN density over NW across medial-lateral axis (Figure 2.7 A) and significant differences between genotypes after surrogacy (SW vs. SH) in lateral sections (Figure 2.7 D; light blue line). S1 was an exception, which showed statistical significance, though the mean density was similar (Table 2.1). This is likely due to differential distribution of PNN density (Figure 2.8).

In the left hemisphere, NH and SH (Figure 2.7 B and 2.7 E, respectively) had significantly higher PNN density in the lateral sections, compared to NW and SW respectively. No significant differences were observed between genotypes in the right hemisphere (Figure 2.7 C, 2.7 F). While analyzing subregions, in the left hemisphere, NH had increased PNN density in S1BF and S1J, compared to NW (Table 2.1, columns 5, 6). SH had increased PNN density in only S1FL, compared to SW (Table 2.1, columns 7, 8). In the right hemisphere, NH had increased PNN density in S1BF, S1J and S1, compared to NW. SH had increased PNN density in S1J and S1, compared to SW. Surrogacy correlated with higher PNN density in only right hemisphere of WT S1BF (NW-R vs. SW-R) (also Fig 2.2 E), while this was not seen in Het (NH vs. SH), with NH-R already exhibiting high PNN density compared to NW-R (Table 2.1, columns 9, 10). S1ULp showed higher PNN density in SH-R compared to NH-R (Table 2.1, columns 10, 12), which was not observed in WT. Taken together, these results suggest that MECP2 regulates dynamic PNN expression, which is then important for appropriate maternal behavior.

Comparing the hemispheres within genotypes, NW and NH did not have significant differences in PNN density in subregions. However, PNN density increased in the right hemisphere of S1FL and S1DZ in both SW and SH (Table 2.1) compared to SW-L and SH-L, respectively. These results suggest a preserved common

experience-dependent plasticity mechanism activation in these regions within the right hemisphere across genotypes in similar social contexts.

Principal component analysis identifies lateral-medial and hemisphere-specific changes in PNN expression

Due to the increasing number of variables being compared, we chose an unsupervised statistical procedure called principal component analysis (PCA), commonly used in genomics/transcriptomics analysis, to determine if patterns emerge from the PNN density data. PCA takes a set of measurements across samples and identifies the measurements that best capture the variation among the samples. It results in a set of uncorrelated components (called principal components) that each capture an orthogonal aspect of the differences across the samples. As input to PCA, we used PNN densities across individual sections and map numbers (represented as lateral coordinates) across all conditions in the five cohorts. If multiple sections per map number were present, values were averaged across sections to give a single density value.

In the first analysis (Figure 2.9 A, B), we sought to determine whether the PNN patterns segregated primarily by cohort or condition (genotype and experience). We preserved data for each individual brain and performed PCA on PNN densities averaged across every set of 2 adjacent map regions. By examining the projection of each individual onto the first and second principal components, we found that, while there is biological variability between cohorts, the individuals in a given cohort did not cluster separately from one another in this unsupervised analysis (Figure 2.9 A, left), suggesting that technical variability in processing samples across five cohorts is not the primary driver of PNN density differences. This is an important control to assess technical or biological variability in this data. Instead, the first principal component (PC1), which explains 40% of the variation in the data (Figure 2.9 A, right, inset), distinguished the SH PNN density patterns from all others, especially NH (Figure 2.9 A, right). We then examined the weights of each brain region in PC1 to determine which regions are most important for capturing the differences between SH and NH. The weights of each section in PC1 (and PC2) shows a left-right asymmetry and

increasing weights for lateral vs. medial sections, confirming our earlier observations (Figure 2.9 B). The findings from our first PCA confirms that variations among mice are resulting from genetic and/or environmental differences and not technical biases. The results further validate our earlier observations of asymmetry in PNN density of left and right hemispheres, augmentation of PNN density in lateral sections, and altered SH PNN density patterns.

In the second PCA (Figure 2.9 C, D), we sought to determine the major PNN density patterns that distinguish genotypes and conditions. Instead of averaging map numbers as in the previous analysis, we preserved data for each map number and averaged across the 5 cohorts for each condition and then performed PCA to determine the major distinguishing patterns. This analysis revealed patterns of PNN density that best distinguish NH vs. SH (PC1) and SW vs. SH (PC2) (Figure 2.9 C, D). These PC patterns also reflect the medial/lateral and left/right asymmetries, thus confirming the anatomical and neurobiological distinctions in the previous figures. Overall, we observe that unsupervised analysis identifies these lateral/medial and left/right asymmetries as the major pattern that characterizes the differences in PNN distribution between experience and genotype.

Individual mice exhibit strong laterality for PNN expression

As the previous data were an aggregate/average of five biological replicates, we were interested in determining if hemispheric biases in PNN density were seen in individual mice. For each mouse, we normalized PNN density of left hemisphere to the right hemisphere (Figure 2.10). In SS1 as a whole, a modest left hemisphere bias was seen in three out of five mice across conditions and genotypes (Figure 2.10A). Higher differences in left hemisphere bias is seen in subregions such as S1BF (Figure 2.10B) and S1ULp (Figure 2.10C). Interestingly, a decrease in the left hemisphere bias was observed in most of the SH mice (Figure 2.10 A-C), suggesting that SH brains have intact plasticity mechanisms that can be triggered by this social maternal experience to overcome the abnormal high PNN density lateralization in NH. Together, these results show that individual mice have differing hemispheric bias in PNN density in

SS1, which may contribute, in a MECP2-dependent manner, to individual variability in responding to and consolidating new tasks involved in tactile sensation.

Mecp2 is a X-linked gene. It is possible that skewed MECP2 expression could lead to the individual variability seen in the NH and SH, though this explanation does not apply to the variability seen in the WT as well. Initial observation of individual sections for all ten NH and SH brains did not show patchy, but salt-and-pepper expression of MECP2, in agreement with other work (Metcalf et al., 2006; Rietveld et al., 2015; and reviewed in Ribeiro and MacDonald, 2020), especially in the age of our mice used in the study (7-12 weeks old). However, we did find left-right asymmetry in MECP2 expression in both NH (Figure 2.11 A'', B'', D'', E'') and SH (Figure 2.12, A'', B'', C'', E'') brains. We also found left-right asymmetry in MECP2 expression in both NW (Figure 2.13, to varying degrees in most animals) and SW (Figure 2.14, A'', B'', E''). Together, these results suggest variable MECP2 expression between hemispheres in the same brain of either genotype (WT, Het) or condition (Naïve, Sur).

Discussion

Given the long-standing and revitalized interest in extracellular matrix structures in the brain, we sought to systematically characterize high-intensity PNN expression in the whole SS1 in a model of adult experience-dependent plasticity, in relevant social behavioral conditions. To our knowledge, this is the first systematic characterization of PNN expression in the adult primary somatosensory cortex with detailed information about subregions and laterality in individual mice.

In early postnatal cortical development, expression of PNNs increases progressively with the maturation of that network. An excellent example is the primary visual cortex where the developmental increase of PNNs is regulated by visual experience (Beurdeley et al., 2012; Hou et al., 2017). PNNs in mature primary visual cortex mainly surround the soma and proximal dendrites of PV+ interneurons (Celio, 1993; Hartig et al., 1992; Ueno et al., 2018). Mature PNNs are thought to be inhibitory for experience-dependent plasticity, as their increase in developing primary visual cortex correlates with the termination of the critical period and PNN removal in adult

primary visual cortex restores plasticity, as measured by ocular dominance plasticity assays (Bavelier et al., 2010; Pizzorusso et al., 2002, 2006). These and studies in other brain regions (amygdala, hippocampus, piriform & auditory cortex) have suggested that PNNs are stable, long-term structures (Miyata and Kitagawa, 2017; Sorg et al., 2016; Ueno et al., 2019). Experiments involving the enzymatic removal of PNN by chondroitinase ABC (ChABC) or hyaluronidase injections in amygdala, hippocampus, piriform and auditory cortices have shown that synaptic plasticity can be reactivated (Banerjee et al., 2017; Gogolla et al., 2009; Kochlamazashvili et al., 2010; Krishnan, Lau et al., 2017; Pizzorusso et al., 2002; Thompson et al., 2018).

A word of caution: due to ease of immunostaining with WFA and manipulation experiments with ChABC, many studies now employ PNNs as markers for plasticity. Our characterization in adult brains shows that these structures are dynamic and have hemisphere- and subregion-specific expression, hinting at potential neural circuitry mechanisms involving laterality in mice. Systematic and careful analysis must be taken to fully characterize PNN expression in experimental design rather than using standard “representative” sample approaches in immunostaining.

What governs PNN dynamics in adults?

Matrix metallopeptides and proteases are known to assist in remodeling extracellular matrix structures (Lorenzo Bozzelli et al., 2018; Lu et al., 2011; Miyata and Kitagawa, 2017). However, the contexts and mechanisms for inducing remodeling in adult brains are currently unclear. Some regional and temporal changes in PNN density have been described before (Ueno et al., 2018, 2019). However, systematic, finer scale whole-brain analysis of WFA expression across entire brain regions during development and adulthood has not been performed. Our study suggests PNNs, as measured by WFA immunostaining, may not be stable and static structures as once thought. In this study, we show that high-intensity PNNs of SS1 exhibit increased and decreased expression in a subregion-specific, hemisphere-specific manner, after maternal behavior experience. Currently, these differences in PNN expression between naïve and surrogate occur over one-two weeks (three – five days before pups are born plus six days of behavior before mice are perfused). The rate of PNN

formation and remodeling, which might ultimately affect tactile perception and efficient pup retrieval, remains unknown.

In the Paxinos and Franklin atlas, anatomical subregions were classified based on structural connectivity studies. Based on these anatomical characterizations, we speculate that changes in PNN density in specific subregions could impact information processing. For example, when NW female mice learn maternal behavior to become surrogates, there is higher PNN density in the right S1BF, with a concomitant lower PNN density in left S1FL (Figure 2.15, colors within IV), suggesting that these changes contribute to solidifying new synaptic contacts in S1BF, while promoting remodeling in S1FL. Together, these changes could ultimately help process new tactile information related to pups and the mother acquired by the whiskers and forelimbs. Furthermore, right hemisphere specific PNN increases in S1FL and S1DZ suggests specific rewiring in subregions that could contribute to efficient information processing associated with laterality and dominant hemispheres (Figure 2.15, arrows within IV).

This idea of lateralization of function in the rodent brain has been a topic of longstanding yet of sporadic interest in the field (Glick and Ross, 1981; Kim et al., 2012; Soma et al., 2017; Wu et al., 2014). However, the underlying cellular mechanisms governing laterality are unknown. Our novel finding that PNNs are expressed in a lateralized manner in mouse SS1 suggests that the lateralized expression could contribute to functional specialization. Pharmacological manipulations of removing PNNs unilaterally in SS1 subregions and exploring the functional consequences via electrophysiological and behavioral studies are ongoing. Previously we showed that, in this adult female mouse model for Rett Syndrome (Het), PNNs were increased in a transient atypical manner in the auditory cortex, which correlated with their inefficient pup retrieval (Krishnan, Lau et al., 2017). Manipulating auditory cortex PNNs by ChABC injections or genetic reductions in Het significantly improved aspects of SH pup retrieval behavior, showing that PNNs play crucial roles in learning and executing this behavior (Krishnan, Lau et al., 2017). We recently showed that aberrant PNN expression leads to dysregulation of auditory cortical PV networks in Het, while behaviorally relevant pup vocalization stimuli were presented during *in vivo* awake

electrophysiological recordings (Lau et al., 2020). Here, we showed that naïve Het already have abnormal increased PNN expression in subregions of SS1 compared to naïve WT (Figure 2.15, II), and surrogacy further increased PNN expressions (Figure 2.15, III). Current results suggest information flow, network activation and multisensory integration could be affected in Het, in specific cortical regions such as SS1 and auditory cortex (Krishnan et al., 2017; Lau et al., 2020; Morello et al., 2018). Further whole brain analysis on laterality in PNN density in the auditory cortex is warranted, especially due to reports suggesting left hemisphere-specific neural circuitry activation (Ehret et al., 1987; Marlin et al., 2015; Stiebler et al., 1997).

What regulates expression of PNN proteins?

Changes in PNN expression in the early developing cortex correlates with the increasing expression of MECP2, PV and other components of the GABAergic machinery in PV+ GABAergic neurons (Krishnan et al., 2015). Expression of MECP2, PV (calcium binding protein), GAD67 (major enzyme that makes GABA in the cortex) and PNNs can dynamically change with activity, in both developing and adult brains (Krishnan et al., 2015, 2017; Ye and Miao, 2013). MECP2 directly occupies the promoter regions of *Gad1* (gene that makes GAD67) and *PV* (Chao et al., 2010; Durand et al., 2012), thus potentially configuring chromatin in these promoter and enhancer regions for appropriate activity- and experience-dependent regulation. MECP2 regulates many genes (Cohen et al., 2011b; Gabel et al., 2015); therefore, genes encoding for PNN proteins could also be regulated by MECP2. Previously, we showed that the expression of mature PNNs is accelerated in the developing male *Mecp2*-null primary visual cortex (Krishnan K, Wang BS et al, 2015), suggesting MECP2 could act as a repressor of PNNs. However, reducing GABAergic inhibition by using a *Gad1* heterozygous allele in *Mecp2*-mutant backgrounds reduced PNN expression to WT levels (Krishnan et al., 2015, 2017), suggesting that expression of the extracellular matrix proteins that ultimately form PNNs may not require direct control of MECP2 and is amenable to change with dynamic inhibition changes.

Mosaicism in MECP2 expression and Het variability

Most previous studies in mouse models of RTT were conducted in *Mecp2*-null male mice, because they exhibit earlier and more severe phenotypes in many standard assays. Therefore, with the exception of a few studies (Garg et al., 2013; Ribeiro and MacDonald, 2020; Samaco et al., 2013; Stearns et al., 2007), the molecular, circuit and behavioral defects in *Mecp2*^{Het} female mice are largely unknown. Since RTT affects predominantly females, *Mecp2*^{Het} female mice represent a more translationally relevant model of RTT than *Mecp2*-null male mice, though they are rarely used in studies due to random X chromosome inactivation patterns contributing to variability in phenotypes. In the alloparental behavioral model, we have shown that even with mosaic expression of MECP2 in the auditory cortex of Het, higher MECP2 expression correlates well with better efficiency during pup gathering behavior (Krishnan, Lau et al., 2017). MECP2 is known to have different levels in different cell types (Lee et al., 2014; Lioy et al., 2011; Rakela et al., 2018; Song et al., 2014). MECP2 protein function can be regulated by activity-dependent post-translational mechanisms (Cohen et al., 2011b; Ebert et al., 2013; Gabel et al., 2015); however, these studies do not take laterality or regional differences into account. Here, we find that MECP2 expression is asymmetric between left and right hemispheres of SS1, in both WT and Het with individual variations across brains (Figures 2.11-14). These results suggest a more in-depth and systematic analysis is required to determine how variable MECP2 expression in hemispheres, subregions and cell types over time could result in diverse phenotypes. Maternal or paternal allele activation in individual cells should also be taken into consideration, as it could contribute to laterality in CNS cell types (Wu et al., 2014).

Increased PNN density in the Naive Mecp2^{Het}

The primary somatosensory cortex, specifically the barrel field cortex, is a well-studied region of the rodent brain, as it integrates tactile information from the body which is important for navigating their environment. The barrel field cortex (S1BF) receives and integrates tactile sensory information from the whiskers (Woolsey, 1988), which is important in social (Bobrov et al., 2014; Lenschow and Brecht, 2015) and

asocial contexts. In an asocial context, whiskers are important for navigating the environment, such as prey capture, elevation navigation and discriminating between textures (Anjum et al., 2006; Arkley et al., 2017; Diamond et al., 2008; Guic-Robles et al., 1989; Park et al., 2020). When somatosensory information from the perioral area (face and whiskers) is removed, this results in a disruption of learned maternal behavior (Morgan et al., 1992).

In the NH brain, we show that there is increased PNN density in the S1BF when compared with the NW (Figure 2.15 II-I). PNNs form as a result of an experience, but the Naïve females have not gone through any distinct experiences, so why is there a difference in PNN expression between the two? As these are adult females, there may be a developmental shift in PNN density within the S1BF. Studies using *Mecp2-null* males found reduced dendritic complexity and smaller barrel cortical representation in the S1BF (Lee et al., 2017; Moroto et al., 2013), similar results were seen in MECP2-cells of the motor cortex of the *Mecp2^{Het}* (Belichenko et al., 2009; Rietveld et al., 2015), though it has not been reported in the S1BF. Due to the mosaic expression of MECP2, there may be neurons within the S1BF with these morphological differences but since all are not affected, the functional defects may take longer to appear. However, this is simply speculation and further studies into the timing of this shift and adult morphological changes in the *Mecp2^{Het}* should be completed.

The atypical increase in PNN density in the S1BF suggests that the NH may have altered tactile perception related to sensory input from the whiskers as PNNs are markers of a mature inhibitory network. This is further supported by studies in *Mecp2-null* males which show that inhibitory signaling is increased in the S1BF of these animals in comparison to WT (Dani et al., 2005; Lee et al., 2017). If the NH has altered tactile perception, which is important for learned maternal behavior, then this could affect the SH's performance in the pup gathering paradigm. A fine-detailed analysis of the pup gathering assay as well as whisker-based tactile assays could shed light on tactile perception deficits in the *Mecp2^{Het}*. Manipulations of the inhibitory network within the S1BF of the Het could rescue the behavioral phenotype, as it did in the auditory

cortex (Krishnan et al., 2017), elucidating a functional relationship between PNNs and behavioral plasticity in this region.

References

- Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* 23, 185–188.
- Anjum, F., Turni, H., Mulder, P.G.H., van der Burg, J., and Brecht, M. (2006). Tactile guidance of prey capture in Etruscan shrews. *Proceedings of the National Academy of Sciences* 103, 16544–16549.
- Arkley, K., Tiktak, G.P., Breakell, V., Prescott, T.J., and Grant, R.A. (2017). Whisker touch guides canopy exploration in a nocturnal, arboreal rodent, the Hazel dormouse (*Muscardinus avellanarius*). *Journal of Comparative Physiology A* 203, 133–142.
- Banerjee, S.B., Gutzeit, V.A., Baman, J., Aoued, H.S., Doshi, N.K., Liu, R.C., and Ressler, K.J. (2017). Perineuronal Nets in the Adult Sensory Cortex Are Necessary for Fear Learning. *Neuron* 95, 169-179.e3.
- Bartos, M., Vida, I., Frotscher, M., Meyer, A., Monyer, H., Geiger, J.R.P., and Jonas, P. (2002). Fast synaptic inhibition promotes synchronized gamma oscillations in hippocampal interneuron networks. *Proc. Natl. Acad. Sci. U.S.A.* 99, 13222–13227.
- Bavelier, D., Levi, D.M., Li, R.W., Dan, Y., and Hensch, T.K. (2010). Removing Brakes on Adult Brain Plasticity: From Molecular to Behavioral Interventions. *Journal of Neuroscience* 30, 14964–14971.
- Beach, F.A., and Jaynes, J. (1956). Studies of Maternal Retrieving in Rats. III. Sensory Cues Involved in the Lactating Female's Response To Her Young. *Behaviour* 10, 104–124.
- Becker, A., Zhang, P., Allmann, L., Meilinger, D., Bertulat, B., Eck, D., Hofstaetter, M., Bartolomei, G., Hottiger, M.O., Schreiber, V., et al. (2016). Poly(ADP-ribosyl)ation of Methyl CpG Binding Domain Protein 2 Regulates Chromatin Structure. *Journal of Biological Chemistry* 291, 4873–4881.
- Begum, M.R., and Sng, J.C.G. (2017). Molecular mechanisms of experience-dependent maturation in cortical GABAergic inhibition. *Journal of Neurochemistry* 142, 649–661.
- Belichenko, N.P., Belichenko, P.V., and Mobley, W.C. (2009). Evidence for both neuronal cell autonomous and nonautonomous effects of methyl-CpG-binding protein 2 in the cerebral cortex of female mice with *Mecp2* mutation. *Neurobiology of Disease* 34, 71–77.
- Bernard, C., and Prochiantz, A. (2016). Otx2-PNN Interaction to Regulate Cortical Plasticity. *Neural Plasticity* 2016, 1–7.

- Berretta, S., Pantazopoulos, H., Markota, M., Brown, C., and Batzianouli, E.T. (2015). Losing the sugar coating: Potential impact of perineuronal net abnormalities on interneurons in schizophrenia. *Schizophrenia Research* 167, 18–27.
- Bignami, A., Asher, R., and Perides, G. (1992). Co-localization of hyaluronic acid and chondroitin sulfate proteoglycan in rat cerebral cortex. *Brain Res.* 579, 173–177.
- Bobrov, E., Wolfe, J., Rao, R.P., and Brecht, M. (2014). The Representation of Social Facial Touch in Rat Barrel Cortex. *Current Biology* 24, 109–115.
- Brecht, M. (2007). Barrel cortex and whisker-mediated behaviors. *Curr. Opin. Neurobiol.* 17, 408–416.
- Brückner, G., Bringmann, A., Köppe, G., Härtig, W., and Brauer, K. (1996). In vivo and in vitro labelling of perineuronal nets in rat brain. *Brain Res.* 720, 84–92.
- Brückner, G., Morawski, M., and Arendt, T. (2008). Aggrecan-based extracellular matrix is an integral part of the human basal ganglia circuit. *Neuroscience* 151, 489–504.
- Cabungcal, J.-H., Steullet, P., Morishita, H., Kraftsik, R., Cuenod, M., Hensch, T.K., and Do, K.Q. (2013). Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proceedings of the National Academy of Sciences* 110, 9130–9135.
- Carstens, K.E., Phillips, M.L., Pozzo-Miller, L., Weinberg, R.J., and Dudek, S.M. (2016). Perineuronal Nets Suppress Plasticity of Excitatory Synapses on CA2 Pyramidal Neurons. *The Journal of Neuroscience* 36, 6312–6320.
- Carulli, D., Rhodes, K.E., and Fawcett, J.W. (2007). Upregulation of aggrecan, link protein 1, and hyaluronan synthases during formation of perineuronal nets in the rat cerebellum. *The Journal of Comparative Neurology* 501, 83–94.
- Cattaud, V., Bezzina, C., Rey, C.C., Lejards, C., Dahan, L., and Verret, L. (2018). Early disruption of parvalbumin expression and perineuronal nets in the hippocampus of the Tg2576 mouse model of Alzheimer's disease can be rescued by enriched environment. *Neurobiol. Aging* 72, 147–158.
- Celio, M.R. (1993). Perineuronal nets of extracellular matrix around parvalbumin-containing neurons of the hippocampus. *Hippocampus* 3 *Spec No*, 55–60.
- Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T.C., Qin, J., and Zoghbi, H.Y. (2008). MeCP2, a Key Contributor to Neurological Disease, Activates and Represses Transcription. *Science* 320, 1224–1229.
- Chao, H.-T., Chen, H., Samaco, R.C., Xue, M., Chahrour, M., Yoo, J., Neul, J.L., Gong, S., Lu, H.-C., Heintz, N., et al. (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* 468, 263–269.
- Chapin, J.K., and Lin, C.S. (1984). Mapping the body representation in the SI cortex of anesthetized and awake rats. *J. Comp. Neurol.* 229, 199–213.
- Cohen, L., Rothschild, G., and Mizrahi, A. (2011a). Multisensory integration of natural odors and sounds in the auditory cortex. *Neuron* 72, 357–369.

Cohen, S., Gabel, H.W., Hemberg, M., Hutchinson, A.N., Sadacca, L.A., Ebert, D.H., Harmin, D.A., Greenberg, R.S., Verdine, V.K., Zhou, Z., et al. (2011b). Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. *Neuron* 72, 72–85.

Dani, V.S., Chang, Q., Maffei, A., Turrigiano, G.G., Jaenisch, R., and Nelson, S.B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 102, 12560–12565.

Deepa, S.S., Umehara, Y., Higashiyama, S., Itoh, N., and Sugahara, K. (2002). Specific molecular interactions of oversulfated chondroitin sulfate E with various heparin-binding growth factors. Implications as a physiological binding partner in the brain and other tissues. *J. Biol. Chem.* 277, 43707–43716.

Diamond, M.E., von Heimendahl, M., and Arabzadeh, E. (2008). Whisker-Mediated Texture Discrimination. *PLoS Biology* 6, e220.

Dityatev, A., Brückner, G., Dityateva, G., Grosche, J., Kleene, R., and Schachner, M. (2007). Activity-dependent formation and functions of chondroitin sulfate-rich extracellular matrix of perineuronal nets. *Developmental Neurobiology* 67, 570–588.

Donato, F., Rompani, S.B., and Caroni, P. (2013). Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* 504, 272–276.

Durand, S., Patrizi, A., Quast, K.B., Hachigian, L., Pavlyuk, R., Saxena, A., Carninci, P., Hensch, T.K., and Fagiolini, M. (2012). NMDA receptor regulation prevents regression of visual cortical function in the absence of *Mecp2*. *Neuron* 76, 1078–1090.

Ebert, D.H., Gabel, H.W., Robinson, N.D., Kastan, N.R., Hu, L.S., Cohen, S., Navarro, A.J., Lyst, M.J., Ekiert, R., Bird, A.P., et al. (2013). Activity-dependent phosphorylation of MeCP2 threonine 308 regulates interaction with NCoR. *Nature* 499, 341–345.

Ehret, G., Koch, M., Haack, B., and Markl, H. (1987). Sex and parental experience determine the onset of an instinctive behavior in mice. *Naturwissenschaften* 74, 47.

Enwright, J.F., Sanapala, S., Foglio, A., Berry, R., Fish, K.N., and Lewis, D.A. (2016). Reduced Labeling of Parvalbumin Neurons and Perineuronal Nets in the Dorsolateral Prefrontal Cortex of Subjects with Schizophrenia. *Neuropsychopharmacology* 41, 2206–2214.

Feldman, D.E., and Brecht, M. (2005). Map plasticity in somatosensory cortex. *Science* 310, 810–815.

Frischknecht, R., Heine, M., Perrais, D., Seidenbecher, C.I., Choquet, D., and Gundelfinger, E.D. (2009). Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. *Nat. Neurosci.* 12, 897–904.

Gabel, H.W., Kinde, B., Stroud, H., Gilbert, C.S., Harmin, D.A., Kastan, N.R., Hemberg, M., Ebert, D.H., and Greenberg, M.E. (2015). Disruption of DNA-methylation-dependent long gene repression in Rett syndrome. *Nature* 522, 89–93.

- Galindo-Leon, E.E., Lin, F.G., and Liu, R.C. (2009). Inhibitory plasticity in a lateral band improves cortical detection of natural vocalizations. *Neuron* 62, 705–716.
- Galtrey, C.M., Asher, R.A., Nothias, F., and Fawcett, J.W. (2007). Promoting plasticity in the spinal cord with chondroitinase improves functional recovery after peripheral nerve repair. *Brain* 130, 926–939.
- Garg, S.K., Lioy, D.T., Cheval, H., McGann, J.C., Bissonnette, J.M., Murtha, M.J., Foust, K.D., Kaspar, B.K., Bird, A., and Mandel, G. (2013). Systemic Delivery of MeCP2 Rescues Behavioral and Cellular Deficits in Female Mouse Models of Rett Syndrome. *Journal of Neuroscience* 33, 13612–13620.
- Glick, S.D., and Ross, D.A. (1981). Lateralization of function in the rat brain. *Trends in Neurosciences* 4, 196–199.
- Gogolla, N., Caroni, P., Lüthi, A., and Herry, C. (2009). Perineuronal nets protect fear memories from erasure. *Science* 325, 1258–1261.
- Guić-Robles, E., Valdivieso, C., and Guajardo, G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. *Behavioural Brain Research* 31, 285–289.
- Gundelfinger, E.D., Frischknecht, R., Choquet, D., and Heine, M. (2010). Converting juvenile into adult plasticity: a role for the brain's extracellular matrix: Converting juvenile into adult plasticity. *European Journal of Neuroscience* 31, 2156–2165.
- Guy, J., Hendrich, B., Holmes, M., Martin, J.E., and Bird, A. (2001). A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat. Genet.* 27, 322–326.
- Hartig, W., Brauer, K., and Bruckner, G. (1992). Wisteria floribunda agglutinin-labelled nets surround parvalbumin-containing neurons. *Neuroreport* 3, 869–872.
- Hou, X., Yoshioka, N., Tsukano, H., Sakai, A., Miyata, S., Watanabe, Y., Yanagawa, Y., Sakimura, K., Takeuchi, K., Kitagawa, H., et al. (2017). Chondroitin Sulfate Is Required for Onset and Offset of Critical Period Plasticity in Visual Cortex. *Scientific Reports* 7.
- Kalemaki, K., Konstantoudaki, X., Tivodar, S., Sidiropoulou, K., and Karagogeos, D. (2018). Mice With Decreased Number of Interneurons Exhibit Aberrant Spontaneous and Oscillatory Activity in the Cortex. *Frontiers in Neural Circuits* 12.
- Kenyon, P., Cronin, P., and Keeble, S. (1981). Disruption of maternal retrieving by perioral anesthesia. *Physiology & Behavior* 27, 313–321.
- Kim, S., Matyas, F., Lee, S., Acsady, L., and Shin, H.-S. (2012). Lateralization of observational fear learning at the cortical but not thalamic level in mice. *Proceedings of the National Academy of Sciences* 109, 15497–15501.
- Kochlamazashvili, G., Henneberger, C., Bukalo, O., Dvoretzkova, E., Senkov, O., Lievens, P.M.-J., Westenbroek, R., Engel, A.K., Catterall, W.A., Rusakov, D.A., et al. (2010). The Extracellular Matrix Molecule Hyaluronic Acid Regulates Hippocampal

Synaptic Plasticity by Modulating Postsynaptic L-Type Ca^{2+} Channels. *Neuron* 67, 116–128.

Kosaka, T., and Heizmann, C.W. (1989). Selective staining of a population of parvalbumin-containing GABAergic neurons in the rat cerebral cortex by lectins with specific affinity for terminal N-acetylgalactosamine. *Brain Res.* 483, 158–163.

Krishnan, K., Wang, B.S., Lu, J., Wang, L., Maffei, A., Cang, J., and Huang, Z.J. (2015). MeCP2 regulates the timing of critical period plasticity that shapes functional connectivity in primary visual cortex. *Proceedings of the National Academy of Sciences of the United States of America* 112, E4782-91.

Krishnan, K., Lau, B.Y.B., Ewall, G., Huang, Z.J., and Shea, S.D. (2017). MECP2 regulates cortical plasticity underlying a learned behaviour in adult female mice. *Nature Communications* 8, 14077.

Kwok, J.C.F., Carulli, D., and Fawcett, J.W. (2010). In vitro modeling of perineuronal nets: hyaluronan synthase and link protein are necessary for their formation and integrity: Hyaluronan synthase and link protein in PNNs. *Journal of Neurochemistry* no-no.

Lagger, S., Connelly, J.C., Schweikert, G., Webb, S., Selfridge, J., Ramsahoye, B.H., Yu, M., He, C., Sanguinetti, G., Sowers, L.C., et al. (2017). MeCP2 recognizes cytosine methylated tri-nucleotide and di-nucleotide sequences to tune transcription in the mammalian brain. *PLoS Genet* 13.

Lau, B.Y.B., Krishnan, K., Huang, Z.J., and Shea, S.D. (2020). Maternal Experience-Dependent Cortical Plasticity in Mice Is Circuit- and Stimulus-Specific and Requires MECP2. *The Journal of Neuroscience* 40, 1514–1526.

Layo, D.E., Lau, B.Y.B., and Krishnan, K. (2020). Mapping and Counting High-Intensity Perineuronal Nets in the Somatosensory Cortex v2 (protocols.io.bcf8itrw).

Lee, T., and Kim, U. (2012). Descending projections from the dysgranular zone of rat primary somatosensory cortex processing deep somatic input. *J. Comp. Neurol.* 520, 1021–1046.

Lee, L.-J., Tsytsarev, V., and Erzurumlu, R.S. (2017). Structural and functional differences in the barrel cortex of *Mecp2* null mice. *Journal of Comparative Neurology* 525, 3951–3961.

Lee, W., Yun, J.-M., Woods, R., Dunaway, K., Yasui, D.H., Lasalle, J.M., and Gong, Q. (2014). MeCP2 regulates activity-dependent transcriptional responses in olfactory sensory neurons. *Human Molecular Genetics* 23, 6366–6374.

Lenschow, C., and Brecht, M. (2015). Barrel Cortex Membrane Potential Dynamics in Social Touch. *Neuron* 85, 718–725.

Lioy, D.T., Garg, S.K., Monaghan, C.E., Raber, J., Foust, K.D., Kaspar, B.K., Hirrlinger, P.G., Kirchhoff, F., Bissonnette, J.M., Ballas, N., et al. (2011). A role for glia in the progression of Rett's syndrome. *Nature* 475, 497–500.

- Lonstein, J.S., and Stern, J.M. (1997). Somatosensory contributions to c-fos activation within the caudal periaqueductal gray of lactating rats: effects of perioral, rooting, and suckling stimuli from pups. *Horm Behav* 32, 155–166.
- Lonstein, J.S., Lévy, F., and Fleming, A.S. (2015). Common and divergent psychobiological mechanisms underlying maternal behaviors in non-human and human mammals. *Hormones and Behavior* 73, 156–185.
- Lorenzo Bozzelli, P., Alaiyed, S., Kim, E., Villapol, S., and Conant, K. (2018). Proteolytic Remodeling of Perineuronal Nets: Effects on Synaptic Plasticity and Neuronal Population Dynamics. *Neural Plasticity* 2018, 5735789.
- Lu, P., Takai, K., Weaver, V.M., and Werb, Z. (2011). Extracellular Matrix Degradation and Remodeling in Development and Disease. *Cold Spring Harbor Perspectives in Biology* 3, a005058–a005058.
- Marlin, B.J., Mitre, M., D’amour, J.A., Chao, M.V., and Froemke, R.C. (2015). Oxytocin enables maternal behaviour by balancing cortical inhibition. *Nature* 520, 499–504.
- Metcalf, B.M., Mullaney, B.C., Johnston, M.V., and Blue, M.E. (2006). Temporal shift in methyl-CpG binding protein 2 expression in a mouse model of Rett syndrome. *Neuroscience* 139, 1449–1460.
- Miyata, S., and Kitagawa, H. (2017). Formation and remodeling of the brain extracellular matrix in neural plasticity: Roles of chondroitin sulfate and hyaluronan. *Biochimica et Biophysica Acta* 1861, 2420–2434.
- Miyata, S., Komatsu, Y., Yoshimura, Y., Taya, C., and Kitagawa, H. (2012). Persistent cortical plasticity by upregulation of chondroitin 6-sulfation. *Nat. Neurosci.* 15, 414–422, S1-2.
- Morawski, M., Brückner, M.K., Riederer, P., Brückner, G., and Arendt, T. (2004). Perineuronal nets potentially protect against oxidative stress. *Exp. Neurol.* 188, 309–315.
- Morello, N., Schina, R., Pilotto, F., Phillips, M., Melani, R., Plicato, O., Pizzorusso, T., Pozzo-Miller, L., and Giustetto, M. (2018). Loss of Mecp2 Causes Atypical Synaptic and Molecular Plasticity of Parvalbumin-Expressing Interneurons Reflecting Rett Syndrome–Like Sensorimotor Defects. *Eneuro* 5, ENEURO.0086-18.2018.
- Morgan, H.D., Fleming, A.S., and Stern, J.M. (1992). Somatosensory control of the onset and retention of maternal responsiveness in primiparous Sprague-Dawley rats. *Physiology & Behavior* 51, 549–555.
- Moroto, M., Nishimura, A., Morimoto, M., Isoda, K., Morita, T., Yoshida, M., Morioka, S., Tozawa, T., Hasegawa, T., Chiyonobu, T., et al. (2013). Altered somatosensory barrel cortex refinement in the developing brain of Mecp2-null mice. *Brain Research* 1537, 319–326.
- Muotri, A.R., Marchetto, M.C.N., Coufal, N.G., Oefner, R., Yeo, G., Nakashima, K., and Gage, F.H. (2010). L1 retrotransposition in neurons is modulated by MeCP2. *Nature* 468, 443–446.

- Murthy, S., Kane, G.A., Katchur, N.J., Lara Mejia, P.S., Obiofuma, G., Buschman, T.J., McEwen, B.S., and Gould, E. (2019). Perineuronal Nets, Inhibitory Interneurons, and Anxiety-Related Ventral Hippocampal Neuronal Oscillations Are Altered by Early Life Adversity. *Biol. Psychiatry* 85, 1011–1020.
- Nakagawa, F., Schulte, B.A., Wu, J.Y., and Spicer, S.S. (1986). GABAergic neurons of rodent brain correspond partially with those staining for glycoconjugate with terminal N-acetylgalactosamine. *J. Neurocytol.* 15, 389–396.
- Noutel, J., Hong, Y.K., Leu, B., Kang, E., and Chen, C. (2011). Experience-dependent retinogeniculate synapse remodeling is abnormal in MeCP2-deficient mice. *Neuron* 70, 35–42.
- Orlando, C., Ster, J., Gerber, U., Fawcett, J.W., and Raineteau, O. (2012). Perisynaptic Chondroitin Sulfate Proteoglycans Restrict Structural Plasticity in an Integrin-Dependent Manner. *Journal of Neuroscience* 32, 18009–18017.
- Pantazopoulos, H., Markota, M., Jaquet, F., Ghosh, D., Wallin, A., Santos, A., Caterson, B., and Berretta, S. (2015). Aggrecan and chondroitin-6-sulfate abnormalities in schizophrenia and bipolar disorder: a postmortem study on the amygdala. *Translational Psychiatry* 5, e496–e496.
- Park, J.M., Hong, Y.K., Rodgers, C.C., Dahan, J.B., Schmidt, E.R., and Bruno, R.M. (2020). Deep and superficial layers of the primary somatosensory cortex are critical for whisker-based texture discrimination in mice (*Neuroscience*).
- Paxinos, G., and Franklin, K.B.J. (2013). Paxino's and Franklin's The Mouse Brain In Stereotaxic Coordinates, 4th Ed (Waltham, MA: Academic Press).
- Picard, N., and Fagiolini, M. (2019). MeCP2: an epigenetic regulator of critical periods. *Curr. Opin. Neurobiol.* 59, 95–101.
- Pizzo, R., Gurgone, A., Castroflorio, E., Amendola, E., Gross, C., Sassoè-Pognetto, M., and Giustetto, M. (2016). Lack of Cdkl5 Disrupts the Organization of Excitatory and Inhibitory Synapses and Parvalbumin Interneurons in the Primary Visual Cortex. *Front Cell Neurosci* 10, 261.
- Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J.W., and Maffei, L. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. *Science* 298, 1248–1251.
- Pizzorusso, T., Medini, P., Landi, S., Baldini, S., Berardi, N., and Maffei, L. (2006). Structural and functional recovery from early monocular deprivation in adult rats. *Proceedings of the National Academy of Sciences* 103, 8517–8522.
- Rakela, B., Brehm, P., and Mandel, G. (2018). Astrocytic modulation of excitatory synaptic signaling in a mouse model of Rett syndrome. *ELife* 7.
- Reinhard, S.M., Rais, M., Afroz, S., Hanania, Y., Pendi, K., Espinoza, K., Rosenthal, R., Binder, D.K., Ethell, I.M., and Razak, K.A. (2019). Reduced perineuronal net expression in Fmr1 KO mice auditory cortex and amygdala is linked to impaired fear-associated memory. *Neurobiol Learn Mem* 164, 107042.

Ribeiro, M.C., and MacDonald, J.L. (2020). Sex differences in Mecp2-mutant Rett syndrome model mice and the impact of cellular mosaicism in phenotype development. *Brain Research* 1729, 146644.

Rietveld, L., Stuss, D.P., McPhee, D., and Delaney, K.R. (2015). Genotype-specific effects of Mecp2 loss-of-function on morphology of Layer V pyramidal neurons in heterozygous female Rett syndrome model mice. *Frontiers in Cellular Neuroscience* 9.

Samaco, R.C., McGraw, C.M., Ward, C.S., Sun, Y., Neul, J.L., and Zoghbi, H.Y. (2013). Female Mecp2^{+/-} mice display robust behavioral deficits on two different genetic backgrounds providing a framework for pre-clinical studies. *Human Molecular Genetics* 22, 96–109.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675.

Shin Yim, Y., Park, A., Berrios, J., Lafourcade, M., Pascual, L.M., Soares, N., Yeon Kim, J., Kim, S., Kim, H., Waisman, A., et al. (2017). Reversing behavioural abnormalities in mice exposed to maternal inflammation. *Nature* 549, 482–487.

Sigal, Y.M., Bae, H., Bogart, L.J., Hensch, T.K., and Zhuang, X. (2019). Structural maturation of cortical perineuronal nets and their perforating synapses revealed by superresolution imaging. *Proc. Natl. Acad. Sci. U.S.A.* 116, 7071–7076.

Skene, P.J., Illingworth, R.S., Webb, S., Kerr, A.R.W., James, K.D., Turner, D.J., Andrews, R., and Bird, A.P. (2010). Neuronal MeCP2 Is Expressed at Near Histone-Octamer Levels and Globally Alters the Chromatin State. *Molecular Cell* 37, 457–468.

Soma, S., Saiki, A., Yoshida, J., Ríos, A., Kawabata, M., Sakai, Y., and Isomura, Y. (2017). Distinct Laterality in Forelimb-Movement Representations of Rat Primary and Secondary Motor Cortical Neurons with Intratelencephalic and Pyramidal Tract Projections. *The Journal of Neuroscience* 37, 10904–10916.

Song, C., Feodorova, Y., Guy, J., Peichl, L., Jost, K., Kimura, H., Cardoso, M., Bird, A., Leonhardt, H., Joffe, B., et al. (2014). DNA methylation reader MECP2: cell type- and differentiation stage-specific protein distribution. *Epigenetics & Chromatin* 7, 17.

Sorg, B.A., Berretta, S., Blacktop, J.M., Fawcett, J.W., Kitagawa, H., Kwok, J.C.F., and Miquel, M. (2016). Casting a Wide Net: Role of Perineuronal Nets in Neural Plasticity. *The Journal of Neuroscience* 36, 11459–11468.

Stearns, N.A., Schaevitz, L.R., Bowling, H., Nag, N., Berger, U.V., and Berger-Sweeney, J. (2007). Behavioral and anatomical abnormalities in Mecp2 mutant mice: A model for Rett syndrome. *Neuroscience* 146, 907–921.

Stern, J.M. (1996). Somatosensation and Maternal Care in Norway Rats. In *Advances in the Study of Behavior*, (Elsevier), pp. 243–294.

Stiebler, I., Neulist, R., Fichtel, I., and Ehret, G. (1997). The auditory cortex of the house mouse: left-right differences, tonotopic organization and quantitative analysis of frequency representation. *J. Comp. Physiol. A* 181, 559–571.

Stolzenberg, D.S., and Champagne, F.A. (2016). Hormonal and non-hormonal bases of maternal behavior: The role of experience and epigenetic mechanisms. *Horm Behav* 77, 204–210.

Sugiyama, S., Prochiantz, A., and Hensch, T.K. (2009). From brain formation to plasticity: Insights on Otx2 homeoprotein: Otx2 transfer shapes postnatal circuits. *Development, Growth & Differentiation* 51, 369–377.

Suttkus, A., Rohn, S., Jäger, C., Arendt, T., and Morawski, M. (2012). Neuroprotection against iron-induced cell death by perineuronal nets - an in vivo analysis of oxidative stress. *Am J Neurodegener Dis* 1, 122–129.

Thompson, E.H., Lensjø, K.K., Wigestrang, M.B., Maltre-Sørensen, A., Hafting, T., and Fyhn, M. (2018). Removal of perineuronal nets disrupts recall of a remote fear memory. *Proceedings of the National Academy of Sciences* 115, 607–612.

Ueno, H., Takao, K., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Okamoto, M., Aoki, S., and Ishihara, T. (2018). Age-dependent and region-specific alteration of parvalbumin neurons and perineuronal nets in the mouse cerebral cortex. *Neurochemistry International* 112, 59–70.

Ueno, H., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Matsumoto, Y., Okamoto, M., and Ishihara, T. (2019). Layer-specific expression of extracellular matrix molecules in the mouse somatosensory and piriform cortices. *IBRO Reports* 6, 1–17.

Vo, T., Carulli, D., Ehlert, E.M.E., Kwok, J.C.F., Dick, G., Mecollari, V., Moloney, E.B., Neufeld, G., de Winter, F., Fawcett, J.W., et al. (2013). The chemorepulsive axon guidance protein semaphorin3A is a constituent of perineuronal nets in the adult rodent brain. *Molecular and Cellular Neuroscience* 56, 186–200.

Welker, W., Sanderson, K.J., and Shambes, G.M. (1984). Patterns of afferent projections to transitional zones in the somatic sensorimotor cerebral cortex of albino rats. *Brain Research* 292, 261–267.

de Winter, F., Kwok, J.C.F., Fawcett, J.W., Vo, T.T., Carulli, D., and Verhaagen, J. (2016). The Chemorepulsive Protein Semaphorin 3A and Perineuronal Net-Mediated Plasticity. *Neural Plasticity* 2016, 1–14.

Woolsey, T.A. (1988). Barrels, Vibrissae, and Topographic Representations. In *Sensory Systems: II*, (Boston, MA: Birkhäuser Boston), pp. 7–9.

Wu, H., Luo, J., Yu, H., Rattner, A., Mo, A., Wang, Y., Smallwood, P.M., Erlanger, B., Wheelan, S.J., and Nathans, J. (2014). Cellular Resolution Maps of X Chromosome Inactivation: Implications for Neural Development, Function, and Disease. *Neuron* 81, 103–119.

Ye, Q., and Miao, Q. (2013). Experience-dependent development of perineuronal nets and chondroitin sulfate proteoglycan receptors in mouse visual cortex. *Matrix Biology* 32, 352–363.

Zhou, Z., Hong, E.J., Cohen, S., Zhao, W., Ho, H.H., Schmidt, L., Chen, W.G., Lin, Y., Savner, E., Griffith, E.C., et al. (2006). Brain-Specific Phosphorylation of MeCP2

Regulates Activity-Dependent Bdnf Transcription, Dendritic Growth, and Spine Maturation. *Neuron* 52, 255–269.

Appendix

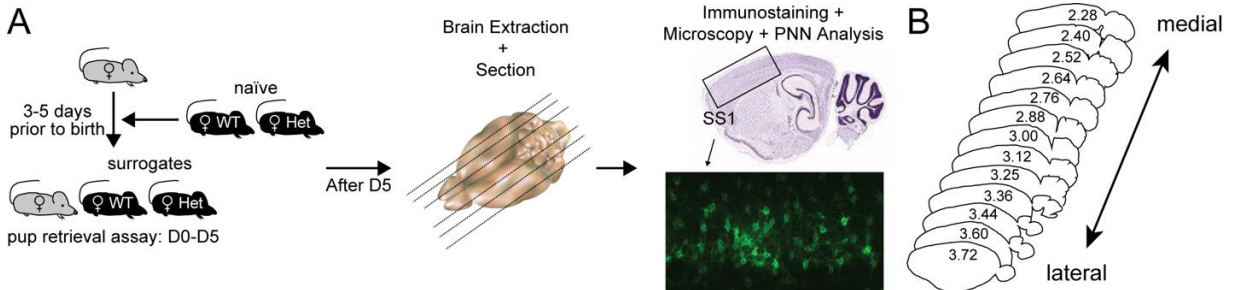
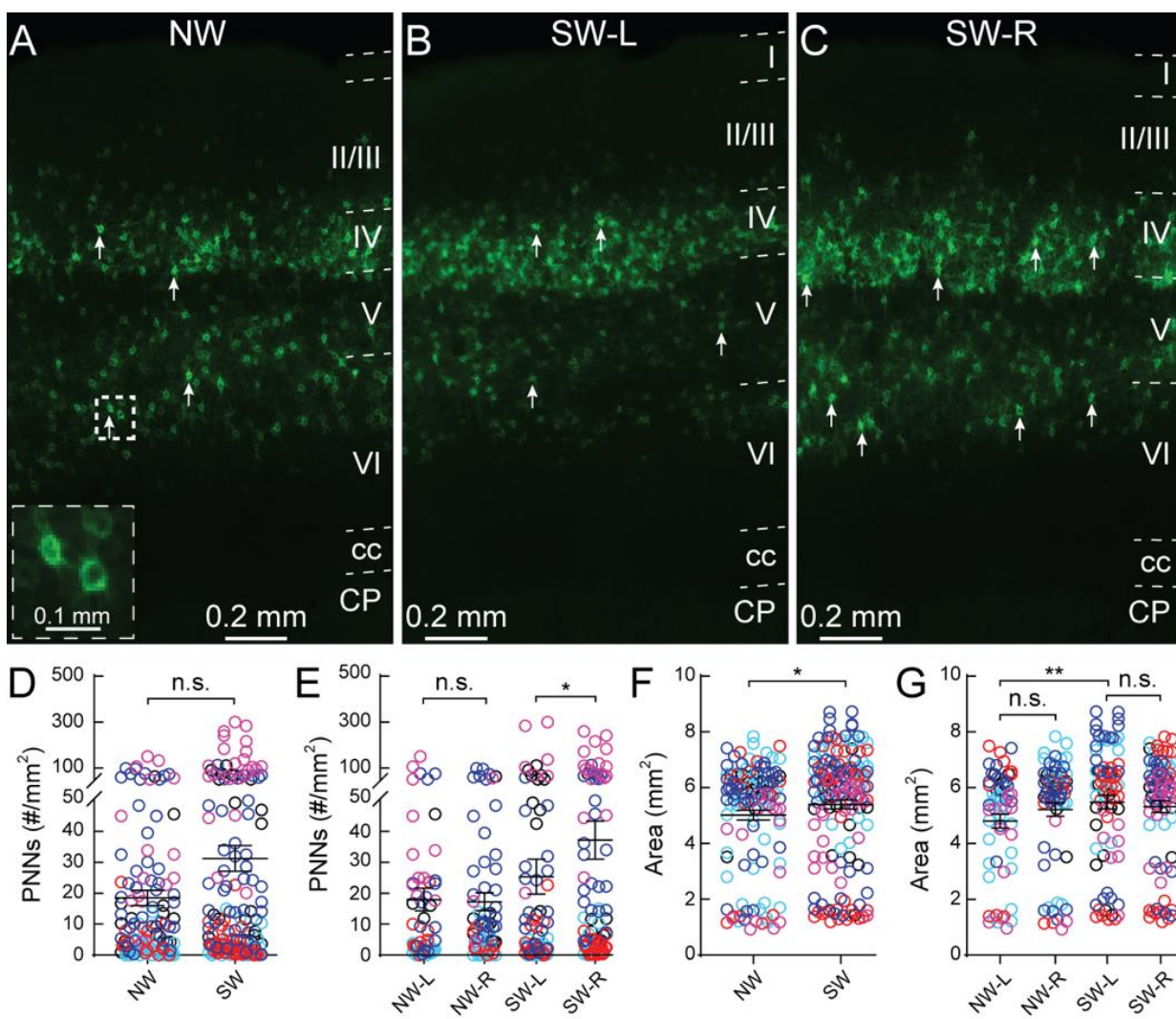


Figure 2.1. Schema representing behavioral and histology pipeline.

(A) (Left) Alloparental behavioral model in mice. Pregnant CBA/CaJ female (grey mouse) is cohoused with adult female naïve WT and naïve Het littermate controls, which changes their status to surrogates, 3-5 days before birth of pups. Once pups are born, pup retrieval assay is performed with the surrogates from day 0 (D0) to 5 (D5). (Center) After behavioral experiments on D5, surrogate mice and age-matched naïve counterparts are perfused, their brains extracted, and sectioned as a single cohort. (Right) Standard immunostaining and imaging with epifluorescent slide scanner are performed, to image and analyze PNNs, as elaborated in Methods. **(B)** Schema of mouse brain sections cut in sagittal orientation, depicting all medial (2.28 mm, corresponding to map number 120) to lateral (3.72 mm, corresponding to map number 132) regions of SS1 analyzed for this study. Coordinate maps are based on Paxinos and Franklin's mouse brain atlas, 4th edition.

Figure 2.2. Surrogate *Mecp2*^{WT} (Wild type) mice exhibit hemisphere specific increase in PNN density in the primary somatosensory cortex (SS1).

(A-C) Representative epifluorescent images of PNN expression in SS1 of naïve WT (NW) (A) as well as left (L) (B) and right (R) (C) hemispheres of surrogate WT (SW). Layers 1 through 6 are outlined. CP = caudate putamen and cc = corpus callosum. Arrows indicate examples of high-intensity PNNs analyzed for the study. Inset in A shows magnified PNN structures in the box. (D) Combined hemisphere analysis of the density of high-intensity PNNs was not significantly different between NW and SW (NW: n = 123 images; SW: n = 162; *Mann-Whitney test*, $p > 0.05$). (E) Separate analysis of left and right hemisphere revealed a significant increase of high-intensity PNNs in the right hemisphere of SW (SW-R; n = 81 images) compared to the left hemisphere of SW (SW-L; n = 81 images) (*Kruskal-Wallis followed by Dunn's test*, $*p < 0.05$), while no significant difference was observed between hemispheres of NW (NW-L: n = 59 images; NW-R: n = 64 images; *Mann-Whitney test*, $p > 0.05$). (F-G) Size of SS1 was overall significantly larger in SW compared to NW (F) (NW: n = 123 images; SW: n = 162 images; *Mann-Whitney test*, $*p < 0.05$). Enlargement of SS1 in SW occurred in the left hemisphere (G) (NW-L: n = 59 images, NW-R: n = 64 images; SW-L: n = 81 images; SW-R: n = 81 images; *Kruskal-Wallis followed by Dunn's test*, $**p < 0.01$). For D-G, n.s. = not significant. Different colors represent each of the five cohorts. Each open circle represents PNN density in an individual brain section.



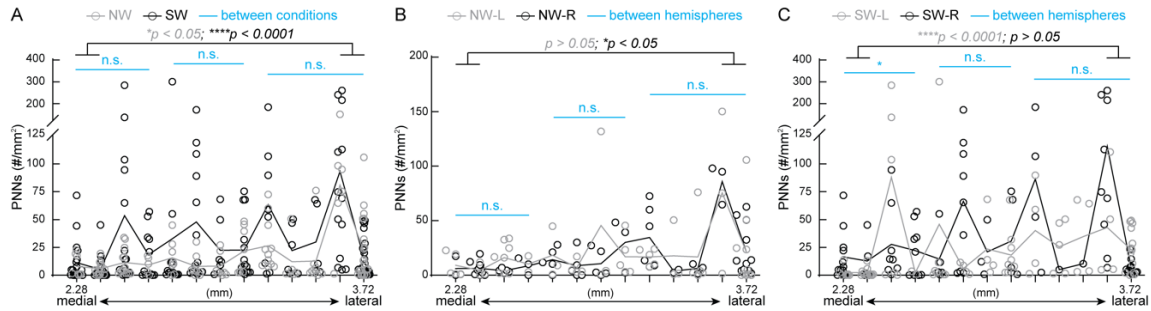
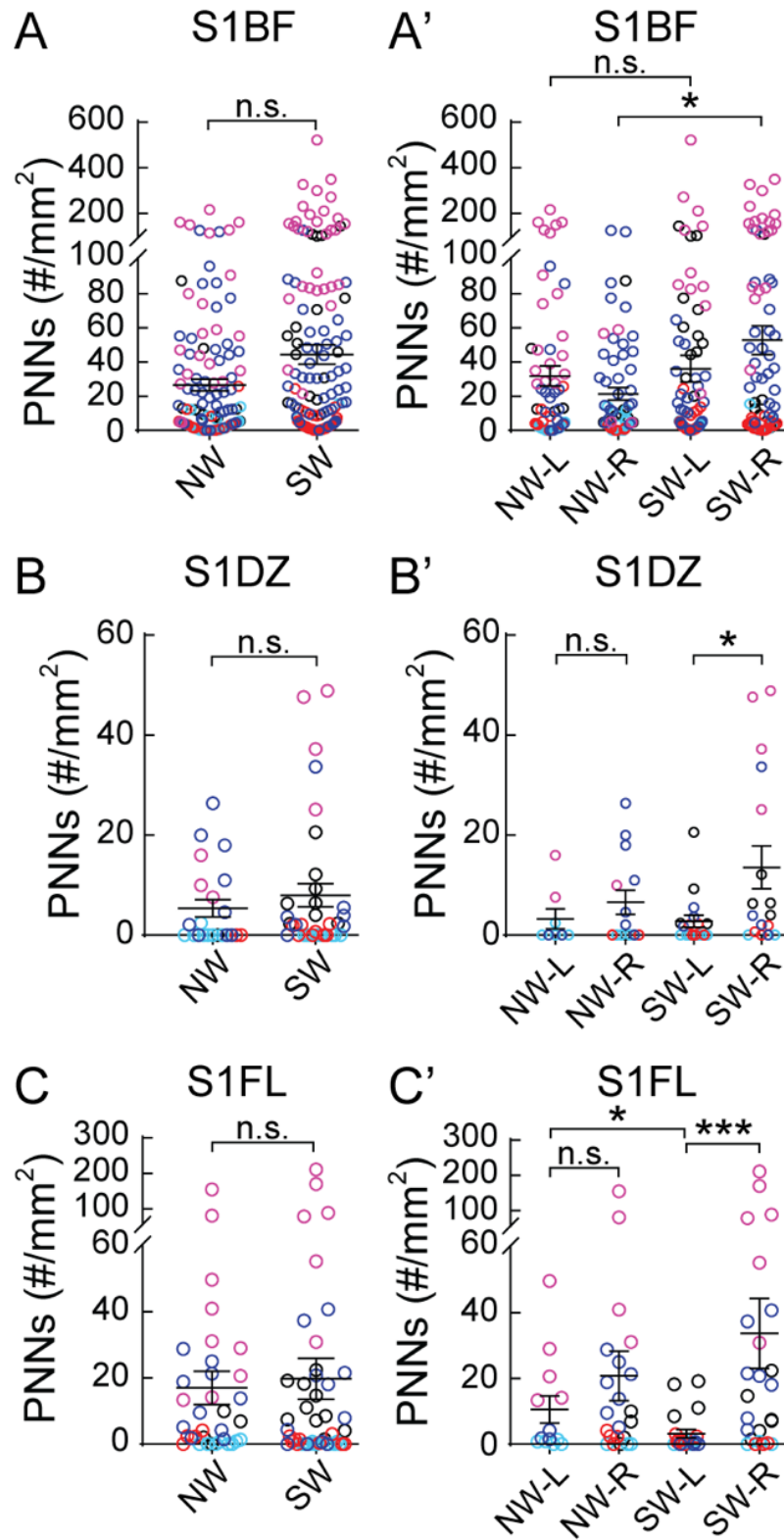


Figure 2.3. Dynamic changes in PNN density across medial-lateral axis of both naïve and surrogate WT SS1.

(A) Distribution of combined PNN density from left and right hemispheres of SS1 revealed an increase of PNN density in lateral sections compared to the medial sections of the same condition. Statistical analysis revealed significant differences between the combined two most medial regions and the combined two most lateral regions within conditions (grey p value denotes NW: $n = 22$ images for medial, 28 images for lateral; black p value denotes SW: $n = 37$ images for medial, 37 images for lateral). This medial-lateral comparison analysis method also applies to all subsequent figure panels. No significant difference in PNN density was found between conditions, represented by the light blue lines. Statistical analysis of the most medial region was a combined of 4 map numbers (NW: $n = 40$ images; SW: $n = 62$ images), middle region was a combined of 4 map numbers (NW: $n = 32$ images; SW: $n = 46$ images) and most lateral region was a combined of 5 map numbers (NW: $n = 52$ images; SW: $n = 56$ images). This sub-regional comparison method also applies to all subsequent figure panels. $N = 5$ -24 images per map number. **(B)** Analysis of the right hemisphere in NW revealed that lateral sections had significantly higher density than the medial sections (NW-R, black p value; $n = 14$ images for medial, 15 images for lateral). In the left hemisphere, NW-L did not show any significant difference in medial-lateral axis (grey p value; $n = 8$ images for medial, 13 images for lateral). Across the subregions in the medial-lateral axis, there was no significant difference between the hemispheres in NW (blue lines; medial-NW: $n = 18$ images for L, 22 images for R; middle-NW: $n = 15$ images for L, 18 images for R; lateral-NW: $n = 26$ images per hemisphere). **(C)** A different pattern of hemisphere-specific differences was observed in SW. SW-R did not have significant differences between medial and lateral sections, while SW-L did (grey p value denotes SW-L: $n = 20$ images for medial, 17 images for lateral; black p value denotes SW-R: $n = 17$ images for medial, 20 images for lateral). While PNN density did not differ between SW-L and SW-R in middle and lateral regions, SW-R exhibited significantly higher PNN density in the medial regions than SW-L (medial-SW: $n = 28$ images for L, 34 images for R; middle-SW: $n = 24$ images for L, 22 images for R; lateral-SW: $n = 30$ images for L, 26 images for R). For A-C, lines represent the mean values. Each dot represents PNN density in an individual section. *Mann-Whitney test*: $*p < 0.05$, *n.s.* = *not significant*, 5 mice per condition. For B and C, $n = 1 - 13$ images per map number.

Figure 2.4. PNN density varies across subregions of WT SS1.

(A-C) Analysis of both hemispheres for SS1 barrel field (S1BF, A), dysgranular zone (S1DZ, B) and forelimb (S1FL, C) revealed no significant differences between NW and SW (S1BF: NW - $n = 131$ images, SW - $n = 168$ images; S1DZ: NW - $n = 22$ images, SW - $n = 35$ images; S1FL: NW - $n = 35$ images, SW - $n = 46$ images; 5 mice per condition; *Mann-Whitney test*, $p > 0.05$, *n.s.* = not significant). **(A'-C')** Analysis of sub regional SS1 by hemispheres revealed dynamic changes in PNN expression. In S1BF (A'), a significant increase of PNN expression was detected in the right hemisphere of SW (SW-R) compared to NW (NW-R) (NW-L: $n = 65$ images; NW-R: $n = 66$ images; SW-L: $n = 85$ images; SW-R: $n = 83$ images). In S1DZ (B'), while no significant difference was observed in PNN density between hemispheres in NW, there was a significant increase of PNN density in the right hemisphere (SW-R) compared to the left (SW-L) (NW-L: $n = 8$ images; NW-R: $n = 14$ images; SW-L: $n = 18$ images; SW-R: $n = 17$ images). A similar pattern of PNN plasticity was also detected in S1FL (C'), where PNN density was significantly increased in the right hemisphere than the left of SW. Interestingly, SW-L exhibited significantly lower PNN density compared to NW-L (Figure 2.4C'; NW-L: $n = 13$ images; NW-R: $n = 22$ images; SW-L: $n = 21$ images; SW-R: $n = 25$ images). For A'-C', 5 mice per condition; *Kruskal-Wallis followed by Dunn's test*, $*p < 0.05$, $***p < 0.001$, *n.s.* = not significant.



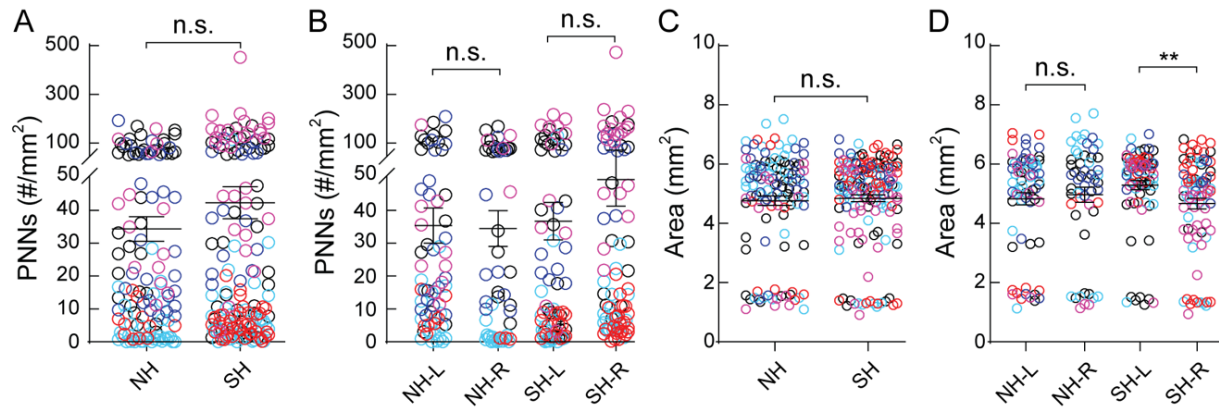


Figure 2.5. Hemisphere-specific PNN density changes are not conserved in *Mecp2*^{Het} after maternal behavior.

(A) Combined hemispheric analysis of PNN density of SS1 did not reveal significant changes between naïve Het (NH, $n = 123$ images) and surrogate Het (SH, $n = 162$ images) (*Mann-Whitney test*, $p > 0.05$). **(B)** Analysis of PNN density between hemispheres of SS1 revealed no significant difference between conditions or within hemispheres of naïve or surrogate Het ($n = 54 - 82$ images; *Kruskal-Wallis followed by Dunn's test*, $p > 0.05$). **(C)** Analysis of SS1 area in both hemispheres revealed no significant changes after maternal learning (NH: $n = 123$ images; SH: $n = 162$ images; *Mann-Whitney test*, $p > 0.05$). **(D)** Area analysis by hemispheres reveal that left hemisphere of SH (SH-L) was significantly larger than the right hemisphere of SH (SH-R). This hemispheric area bias was absent in NH ($n = 54 - 82$ images; *Kruskal-Wallis followed by Dunn's test*, $**p < 0.01$). N.s. = not significant. 5 mice per condition.

Table 2.1. Average high-intensity PNN density across subregions and hemispheres of the SS1 before and after maternal behavior experience.

NW, NH, SW and SH are the four different conditions. Primary somatosensory cortex subregions: S1BF, S1ULp, S1FL, S1J, S1, and S1DZ. Significant differences are denoted between genotypes by shading (E.g., NW vs NH), between hemispheres of the same condition by bold lettering, and between Naïve and Surrogate in the same genotype by **bold borders**. Each letter pair corresponds to statistically significant differences between two conditions. Numbers correspond to average PNN density with standard error mean across multiple sections. N = 8 – 85 images for hemisphere analysis, 123-168 images for combined hemisphere analysis; 5 mice per condition; *Kruskal-Wallis followed by Dunn's test*.

Regions	Whole brain					Left Hemisphere					Right Hemisphere			
	NW	NH	SW	SH		NW-L	NH-L	SW-L	SH-L		NW-R	NH-R	SW-R	SH-R
S1BF	26.6 ± 3.5 ^e	56.0 ± 6.6 ^e	44.4 ± 5.8	47.0 ± 5.4		31.7 ± 5.9 ^f	41.0 ± 5.9 ^f	36.1 ± 7.8	37.6 ± 5.4		21.4 ± 3.6 ^{g,h}	72.2 ± 11.9 ^g	52.8 ± 8.4 ^h	56.3 ± 9.3
S1ULp	28.3 ± 4.8 ^o	47.0 ± 8.1 ^o	37.9 ± 5.7	52.9 ± 7.6		33.4 ± 7.3	45.3 ± 8.2	29.1 ± 6.7	44.2 ± 9.7		23.5 ± 6.3	49.2 ± 15.2 ^p	38.9 ± 8.0	62.5 ± 11.8 ^p
S1FL	17.0 ± 5.0	20.9 ± 4.0	19.8 ± 6.2	18.4 ± 3.6		10.6 ± 4.2 ^s	20.8 ± 5.0	3.2 ± 1.3 ^{s,t,u}	8.1 ± 2.2 ^{t,v}		20.8 ± 7.5	21.0 ± 6.4	33.8 ± 10.7 ^u	30.6 ± 6.6 ^v
S1J	2.5 ± 0.8 ⁱ	4.2 ± 0.9 ^j	6.7 ± 3.6	8.4 ± 2.0		4.0 ± 1.6 ^k	5.2 ± 1.4 ^{k,l}	9.0 ± 6.8	3.6 ± 1.2 ^l		1.3 ± 0.4 ^m	3.0 ± 0.7 ^m	4.2 ± 1.3 ⁿ	14.0 ± 3.9 ⁿ
S1	6.5 ± 0.9 ^a	16.7 ± 2.8 ^a	14.4 ± 3.7 ^b	14.4 ± 2.1 ^b		7.0 ± 1.6	11.4 ± 2.5	16.7 ± 6.6	11.4 ± 2.1		6.0 ± 1.0 ^c	22.5 ± 5.0 ^c	12.3 ± 3.2 ^d	17.5 ± 3.6 ^d
S1DZ	5.4 ± 1.7	9.1 ± 2.6	8.0 ± 2.3	12.7 ± 3.5		3.2 ± 2.0	7.4 ± 3.4	2.8 ± 1.2 ^q	6.7 ± 4.0 ^r		6.6 ± 2.4	10.1 ± 3.8	13.4 ± 4.3 ^q	20.6 ± 5.6 ^r

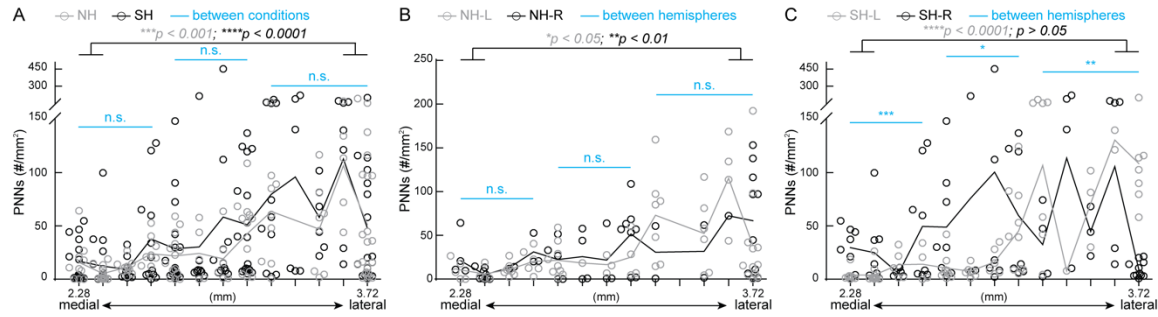


Figure 2.6. Overall patterns of PNN density across medial-lateral axis is preserved in *Mecp2*^{Het}.

(A) Similar to NW-SW in Figure 2.3, NH and SH displayed significant increase of PNN density in lateral sections compared to the medial sections of the same condition (grey p value denotes NH: $n = 23$ images for medial, 27 images for lateral; black p value denotes SH: $n = 36$ images for medial, 25 images for lateral). Along the sub regions encompassing the medial-lateral axis, there was no significant difference between NH and SH, represented by the light blue lines (medial: $n = 42$ images for NH, 59 images for SH; middle: $n = 37$ images for NH, 55 images for SH; lateral: $n = 44$ images for NH, 48 images for SH). $N = 5 - 22$ images per map coordinate. **(B)** Within hemispheres of NH, statistical analysis of the lateral sections of the right (NH-R, black p value) and the left (NH-L, grey p value) showed significantly higher PNN density than their medial sections (NH-L: $n = 9$ images for medial, 16 images for lateral; NH-R: $n = 14$ images for medial, 11 images for lateral). There was no significant difference between hemispheres along the medial-lateral axis (medial: $n = 23$ images for L, 19 images for R; middle: $n = 17$ images for L, 20 images for R; lateral: $n = 29$ images for L, 15 images for R). **(C)** A different pattern of hemisphere-specific differences was observed in SH. SH-R did not display significant difference between medial and lateral sections, while SH-L did (grey p value denotes SH-L: $n = 9$ images in lateral, $n = 20$ images for medial; black p value denotes SH-R: $n = 16$ images for both lateral and medial). Statistical comparison between hemispheres revealed dynamic differences across medial-lateral axis, with SH-R had significantly higher PNN density than SH-L in medial and middle regions, while SH-L had significantly higher PNN density in the lateral regions (light blue lines; medial: $n = 29$ images for L, 30 images for R; middle: $n = 31$ images for L, 24 images for R; lateral: $n = 20$ images for L, 28 images for R). For A-C, lines represent mean values. Each dot represents PNN density in an individual section. *Mann-Whitney test*: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$, *n.s.* = not significant, 5 mice per condition. For B- C, $n = 1 - 12$ images per map coordinate.

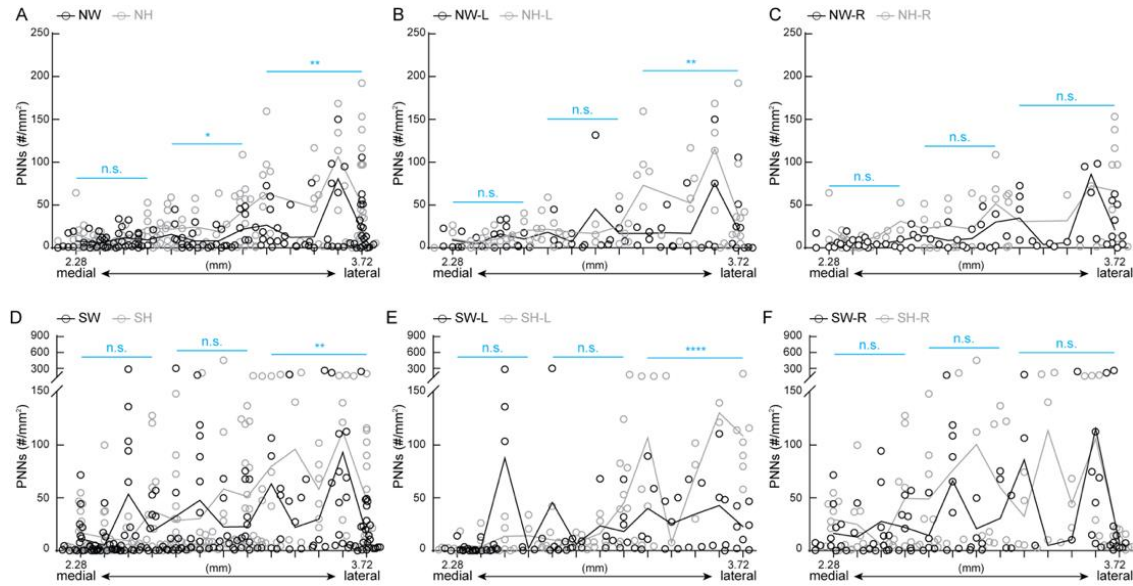


Figure 2.7. Left hemispheres of naïve and surrogate *Mecp2^{Het}* have higher PNN density in the most lateral sections, compared to the wildtype counterparts.

(A) Combined hemispheres of NH had significantly higher PNN density than NW, in the middle and lateral sections. No significant difference was observed in the most medial sections (medial: $n = 40$ images for NW, 42 images for NH; middle: $n = 32$ images for NW, 37 images for NH; lateral: $n = 52$ images for NW, 44 images for NH). **(B)** Comparing left hemisphere PNN density between NW and NH, NH had significantly higher PNN density in the most lateral sections, with no significant differences in the more medial sections (medial: $n = 18$ images for NW-L, 23 images for NH-L; middle: $n = 15$ images for NW-L, 17 images for NH-L; lateral: $n = 26$ images for NW-L, 29 images for NH-L). **(C)** There were no significant differences in the right hemisphere between NW and NH, suggesting the PNN expression in the subregions of the lateral sections of the left hemisphere are particularly dysregulated in NH (medial: $n = 22$ images for NW-R, 19 images for NH-R; middle: $n = 18$ images for NW-R, 20 images for NH-R; lateral: $n = 26$ images for NW-R, 15 images for NH-R). **(D)** SH exhibited higher PNN density over SW only in the most lateral regions (medial: $n = 62$ images for SW, 59 images for SH; middle: $n = 46$ images for SW, 55 images for SH; lateral: $n = 56$ images for SW, 48 images for SH). **(E)** Similar to naïve conditions, only the lateral sections in left hemisphere of SH had significantly higher PNN density than the same regions of SW (medial: $n = 28$ images for SW-L, 29 images for SH-L; middle: $n = 24$ images for SW-L, 31 images for SH-L; lateral: $n = 30$ images for SW-L, 20 images for SH-L). **(F)** No significant differences between SW and SH were found in the right hemisphere (medial: $n = 34$ images for SW-R, 30 images for SH-R; middle: $n = 22$ images for SW-R, 24 images for SH-R; lateral: $n = 26$ images for SW-R, 28 images for SH-R). For A-F, lines represent the mean values. Each dot represents PNN density in an individual section. *Mann-Whitney test*, $*p < 0.05$, $**p < 0.01$, $***p < 0.0001$, *n.s.* = not significant. 5 mice per condition. For A and D, $n = 5$ -24 images per map coordinate. For B-C and E-F, $n = 1$ -13 images per map coordinate.

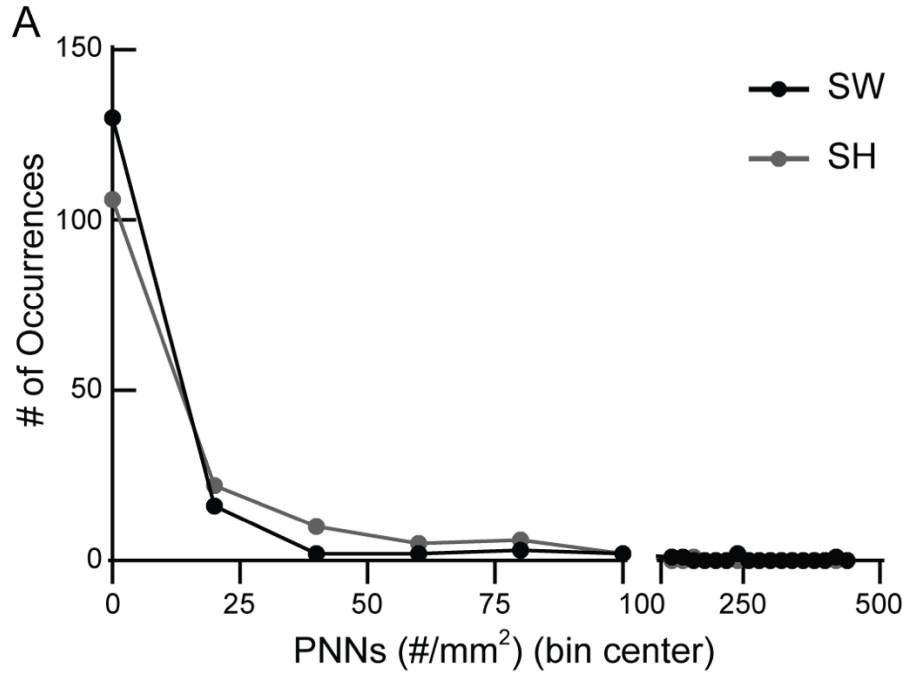
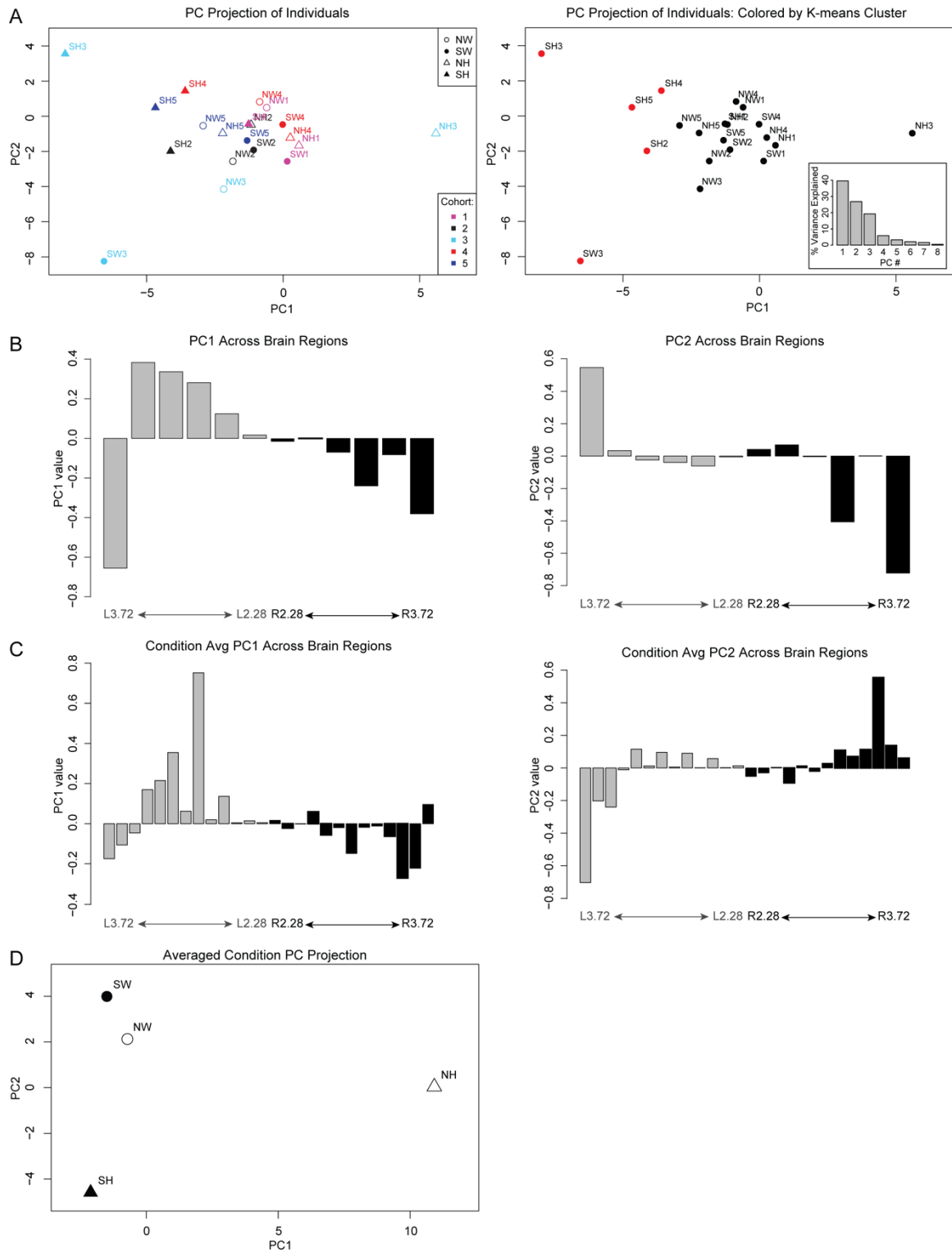


Figure 2.8. Significant difference in S1 PNN density between SH and SW in Table 2.1 due to differential distribution.

(A) Histogram analysis showed that SW had more occurrences of 0 PNN density compared to SH, as shown by black dot (SW) above grey dot (SH). SH had more occurrences of 20-80 PNN density than SW, as shown by grey dots (SH) above black dots (SW). The average PNN density values of SW ($n = 160$ images) and SH ($n = 152$ images) are similar (Table 2.1) (5 mice per condition), but they are statistically different due to this differential distribution of data.

Figure 2.9. Principal component analysis of PNN expression segregates by conditions, lateral/medial axis and hemispheres.

(A) The projection of each individual brain onto PC1 and 2. These PCs are defined by relative weights of the different brain regions – all of the weights for PC1 and 2 are shown in B. (Left) Individuals are colored by cohort with symbol shapes corresponding to their genotype and experience condition. (Right) Individuals are colored by K-means clustering assignment, showing that the primary separation is SH from the rest of the conditions. While PC1 tends to distinguish surrogates from naïve (in particular, surrogate het from all others), the PCA itself is an unbiased analysis that is not designed to calculate the differences in “NH vs. SH”. Rather, the PC1 represents the combination of factors that are the largest source of variation among all the samples. The PC1 itself is fully defined by the weights of the different brain sections. Inset shows the % variance explained by the different PCs, with PC1 explaining the most variance. **(B)** Weights for each brain region for principal component (PC) 1 (left panel) and 2 (right panel) from the analysis in (A). The map regions (corresponding lateral coordinates) with strongest positive and negative values contribute most strongly to the variation between individuals. The weights show a trend from medial to lateral, showing the differences in laterality are the largest source of variation in the data. **(C)** As in B, weights for each brain region for PC1 (left panel) and 2 (right panel) are shown, in this case for PCA on data in which all cohorts were averaged for each condition. **(D)** Conditions projected onto PC1 show a separation of NH from the rest while PC2 axis shows a separation of SH from SW.



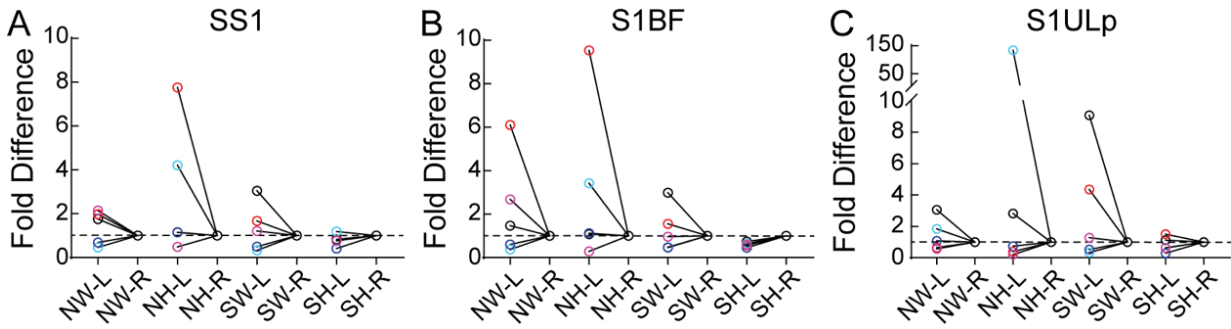


Figure 2.10. Individual brains exhibit differential hemispheric bias in PNN density in specific subregions of SS1.

(A-C) For each brain, PNN density from the left hemisphere was normalized to the right hemisphere. This left-right hemisphere normalization revealed varying patterns of cortical asymmetry. **(A)** In SS1, three NW brains exhibit left hemisphere bias of 2-fold. In SW, left hemisphere bias is seen in 2 brains (black, red). Two NH brains exhibit large fold differences favoring left hemisphere (red, blue circles). In SH, no brains display cortical asymmetry. Similar trends with larger fold differences are seen in S1BF **(B)** and S1ULp **(C)**.

Figure 2.11. Hemispheric bias for MECP2 expression in individual NH brains.

(A-E, A'-E') Representative 20X magnification, tiled projection epifluorescent images showing MECP2 expression in left (A-E) and right (A'-E') SS1 of NH from cohorts 1-5. R = rostral, C = caudal, D = dorsal and V = ventral. (A''-E'') Percentage cumulative frequency distribution of MECP2 intensity within left (grey) and right (black) SS1 of the corresponding NH cohorts. Cohort 1 (A'') expressed more MECP2 in the right hemisphere than the left. Cohorts 2, 4-5 (B'', D''-E'', respectively) expressed more MECP2 in the left hemisphere than the right, while cohort 3 (C'') showed similar MECP2 expression in both hemispheres. A.U. = arbitrary intensity unit. N = 1 image per hemisphere.

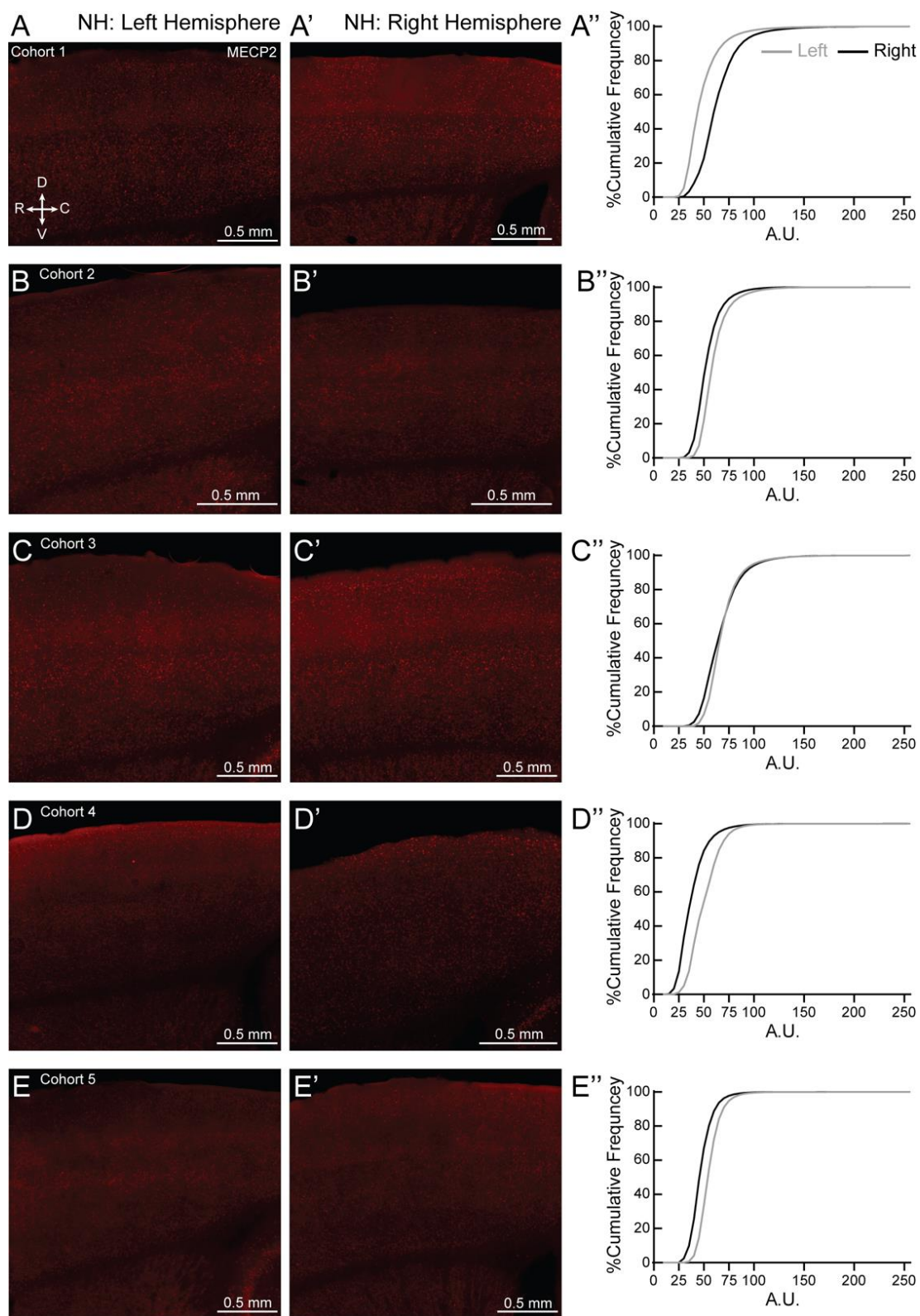


Figure 2.12. Hemispheric bias for MECP2 expression in individual SH brains.

(A-E, A'-E') Representative 20X magnification, tiled projection epifluorescent images showing MECP2 expression in left (A-E) and right (A'-E') SS1 of SH from cohorts 1-5. R = rostral, C = caudal, D = dorsal and V = ventral. **(A''-E'')** Percentage cumulative frequency distribution of MECP2 intensity within left (grey) and right (black) SS1 of the corresponding SH cohorts. Cohort 1 and 3 (A'' and C'', respectively) expressed more MECP2 in the right hemisphere than the left. Cohorts 2 and 5 (B and E'', respectively) expressed slightly more MECP2 in the left hemisphere than the right, while cohort 4 (D'') showed similar MECP2 expression in both hemispheres. A.U. = arbitrary intensity unit. N = 1 image per hemisphere.

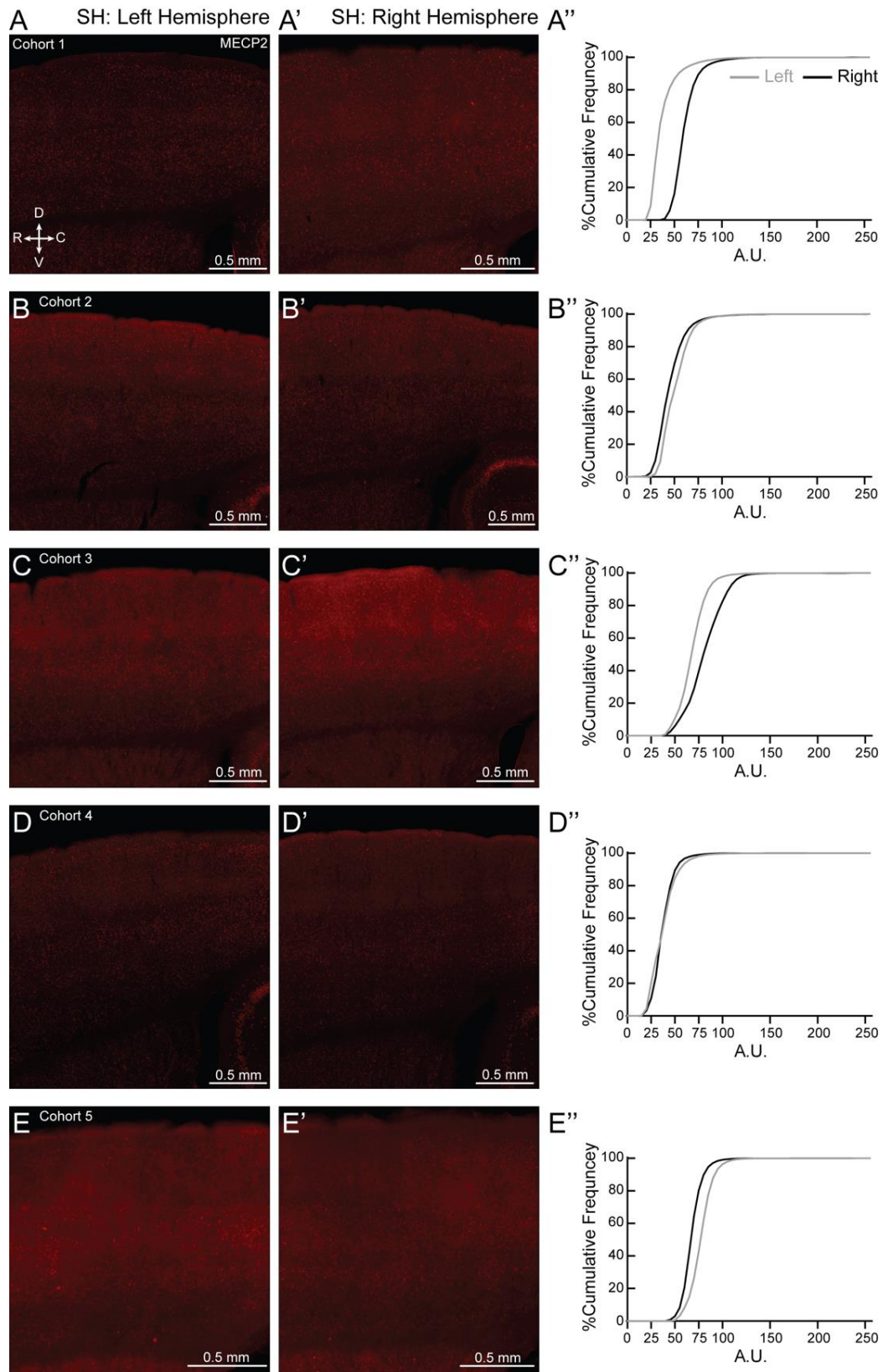


Figure 2.13. Hemispheric bias for MECP2 expression in individual NW brains.

(A-E, A'-E') Representative 20X magnification, tiled projection epifluorescent images showing MECP2 expression in left (A-E) and right (A'-E') SS1 of NW from cohorts 1-5. R = rostral, C = caudal, D = dorsal and V = ventral. **(A''-E'')** Percentage cumulative frequency distribution of MECP2 intensity within left (grey) and right (black) SS1 of the corresponding NW cohorts. Cohort 1 and 4 (A'' and D'', respectively) expressed more MECP2 in the right hemisphere than the left. Cohorts 2, 3 and 5 (B'', C'' and E'', respectively) expressed more MECP2 in the left hemisphere than the right. A.U. = arbitrary intensity unit. N = 1 image per hemisphere.

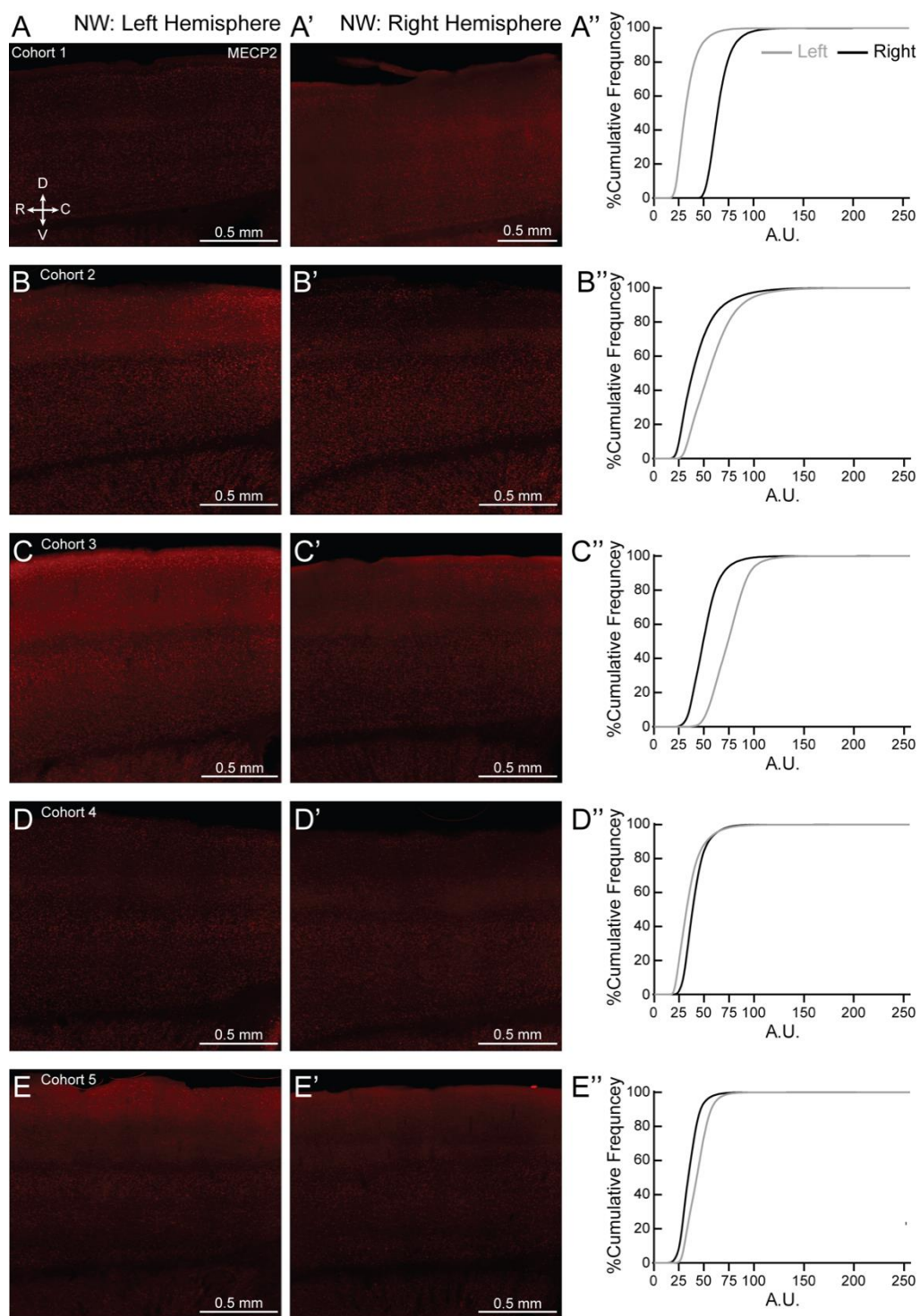
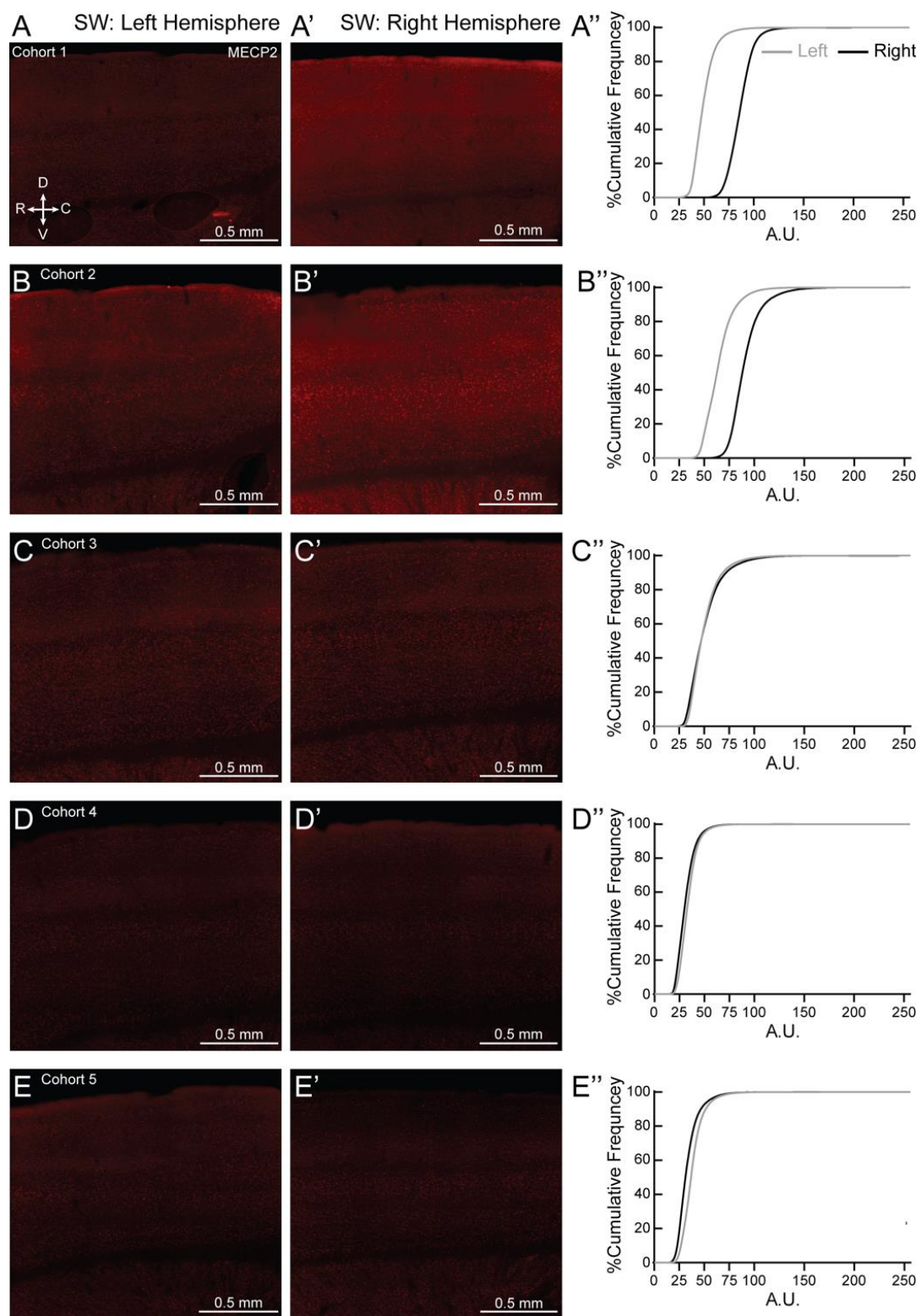


Figure 2.14. Hemispheric bias for MECP2 expression in individual SW brains.

(A-E, A'-E') Representative 20X magnification, tiled projection epifluorescent images showing MECP2 expression in left (A-E) and right (A'-E') SS1 of SW from cohorts 1-5. R = rostral, C = caudal, D = dorsal and V = ventral. **(A''-E'')** Percentage cumulative frequency distribution of MECP2 intensity within left (grey) and right (black) SS1 of the corresponding SW cohorts. Cohort 1 and 2 (A'' and B'', respectively) expressed more MECP2 in the right hemisphere than the left. MECP2 expressions in Cohorts 3-5 (C''-E'') were similar between left and right hemispheres. A.U. = arbitrary intensity unit. N = 1 image per hemisphere.



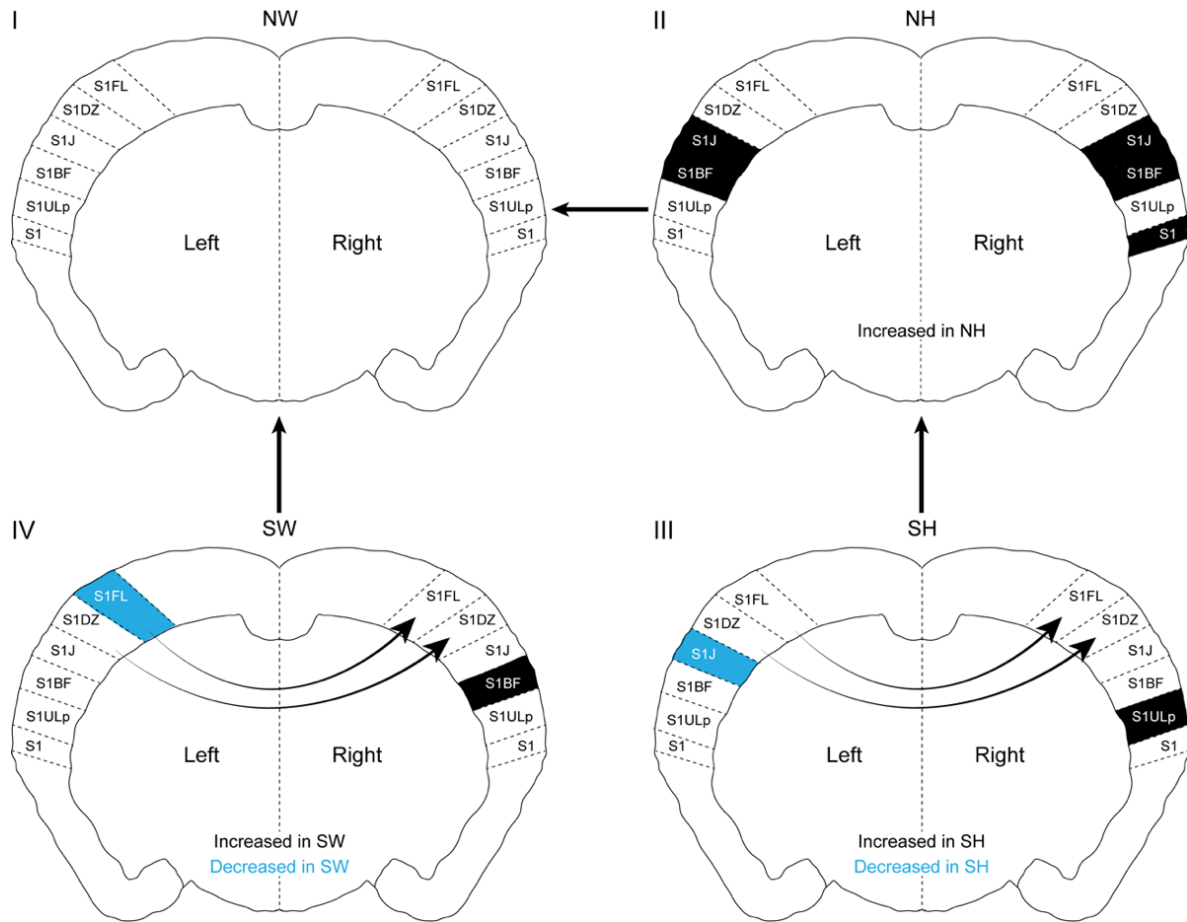


Figure 2.15. Summary of changes in PNN density between genotypes in maternal behavior context.

(Quadrants I-IV) The changes in PNN density (grey and black shading) marked inside the brain slices denote comparisons between conditions connected by outside arrows between brain schemas. Arrows inside the brain schemas indicate hemispheric differences within genotype, with arrowheads pointing to the hemisphere with the higher PNN density. (IV→I) Comparing SW to NW, PNN density is increased in right S1BF (black) and decreased in left S1FL (blue) regions of SW. Within SW, PNN density is higher in the right hemisphere, particularly in S1FL and S1DZ. Taken together, PNN density changes in these particular subregions could contribute to tactile perception in SW, ultimately leading to efficient pup retrieval. (II→I) NH has increased PNN density in specific subregions compared to the NW, suggesting possible tactile perception issues before maternal experience, which could contribute to *Mecp2^{Het}*'s inefficient pup retrieval performance. (III→II) SH has increased PNN density in the right S1ULp (black) and decreased PNN density in left S1J (blue), compared to NH, suggesting possible compensatory plasticity mechanisms after maternal experience in *Mecp2^{Het}*. SH also displays higher right hemisphere PNN density in S1FL and S1DZ than its left hemisphere, similar to SW, suggesting that right hemisphere-specific increases in PNNs in S1FL and S1DZ might be important for processing tactile information during pup retrieval task.

CHAPTER III
IDENTIFICATION OF TACTILE SENSORY DEFICITS IN A FEMALE
MOUSE MODEL OF RETT SYNDROME IN AN AGE- AND CONTEXT-
DEPENDENT MANNER

Abstract

Rett Syndrome (RTT) is a neurological disorder caused by mutations in MECP2, a gene expression regulator. RTT is hypothesized to be a sensory processing disorder, which leads to higher-order cognitive syndromic phenotypes. How MECP2 deficiency causes sensory processing issues is currently unknown. We use a female mouse model of RTT (Het) to study how MECP2 regulates the timing of experience-dependent plasticity during sensory behaviors. Perineuronal nets (PNNs) are extracellular matrix structures that surround parvalbumin + GABAergic neurons in the cortex, marking restricted synaptic plasticity. MECP2 is essential for regulating experience-dependent plasticity, as measured by PNN expression, in the auditory cortex during pup gathering, a complex multisensory behavior. Since pup gathering behavior is multisensory, we chose to look at PNN expression within the primary somatosensory cortex (SS1). Adult Hets perform the pup gathering behavior poorly and show an increase in PNN density within SS1 before (Naïve) and after (Sur) behavioral experience. This suggests that adult NaïveHets are unable to process tactile information causing inefficient pup retrieval.

We developed a series of whisker-based tasks to specifically test tactile perception and found that adult Hets present with deficits in tactile perception (Tac). By time course analysis, we found that 6-week-old (WO) NaïveHets had similar PNN density to Naïve WT, suggesting that a developmental shift after 6 weeks causes dysregulated PNN formation in the Het. The 6 WO Het shows no deficits in tactile perception, when compared to the WT. Interestingly, 6 WO SurHet are inefficient at the pup gathering behavior in comparison to the SurWT. Through whole brain immunohistochemical analysis of the SS1 in 6 WO SurHets, we found 1) PNN density is significantly increased in the SS1 of SurHet compared to SurWT and NaïveHet and 2) PNN density changes are subregion and hemisphere specific. Comparatively, we found subtle PNN density differences between the TacHet and TacWT SS1. Overall, these results suggest that MECP2 influences experience-dependent plasticity within the SS1 during complex, but not simple behaviors. We also identified sensory processing issues within the 'pre-symptomatic' Het, supporting the hypothesis that RTT begins as a sensory processing disorder.

Introduction

Rett Syndrome (RTT) is a neurological disorder characterized by the appearance of higher-order cognitive deficits and motor stereotypies after a period of typical development in childhood, predominantly seen in females (Chahrour and Zoghbi, 2007; Neul et al., 2014). RTT is caused by mutations in MECP2 (methyl-CpG binding protein 2), an activity-dependent gene expression regulator (Amir et al., 1999). MECP2 is important for the function and maintenance of neurons throughout development and into adulthood (Bedogni et al., 2016; Ebert et al., 2013; Kishi and Macklis, 2004; Skene et al., 2010; Stefanelli et al., 2016; Zhou et al., 2006). The delayed onset of RTT syndromic features, along with what is known about MECP2 function, suggests that MECP2 regulates plasticity mechanisms within specified circuits during new experiences (Chahrour et al., 2008; Ebert et al., 2013). Disruption of these plasticity mechanisms could lead to the syndromic phenotypes observed in patients. Based on recent findings that RTT patients begin to develop subtle sensory deficits before onset of RTT syndromic phenotypes (Buchanan et al., 2019; Djukic and Valicenti McDermott, 2012; Nomura, 2005), an emerging hypothesis is that RTT is a sensory processing disorder, which leads to higher-order cognitive, motor, and social communication deficits later in development and into adulthood.

How mutations in MECP2 cause RTT pathogenesis, specifically sensory processing deficiencies, is currently unknown. We hypothesize that MECP2 regulates the timing of experience-dependent plasticity mechanisms within specified circuits during periods of enhanced sensory experience. If mutations in MECP2 are disrupting experience-dependent plasticity, this can prevent developmentally important circuits from forming, which can give rise to behavioral deficits later in life. For example, in the developing visual cortex of mice there is a specific window of time in which visual experience induces plasticity in order to refine binocular vision, called the visual critical period (Fagiolini and Hensch, 2000). This period of time is characterized by the gradual increase in inhibitory markers, such as parvalbumin (PV), GAD67 (enzyme that creates GABA) and perineuronal nets, which then mark the maturation of this circuit and as measured by binocular matching (Fagiolini and Hensch, 2000; Hou et al., 2017).

In *Mecp2*-null male mice, the formation of binocular vision was disrupted by the precocious closure of the visual critical period (Krishnan et al., 2015). This was characterized by the increased expression of PV, the precocious formation of perineuronal nets, and increased inhibitory signaling. This study showed that a lack of MECP2, caused the dysregulation of the cortical inhibitory network during a period of heightened sensory experience, resulting in an underdeveloped binocular visual circuit (Krishnan et al., 2015).

This hypothesis was tested within the context of pup gathering, a complex multisensory paradigm that allows us to test for adult learning and modulation in adult experience-dependent mechanisms in the female *Mecp2*^{Het} (Het). Virgin females with no prior maternal experience (Naive) are able to perform the pup gathering behavior after cohousing with the mother and pups (Sur) (Cohen et al., 2011a; Krishnan et al., 2017). However, the adult SurHet were unable to efficiently perform the pup gathering behaviors due to dysregulated inhibitory networks and an increased expression of perineuronal nets within the auditory cortex (Krishnan et al., 2017; Lau et al., 2020a). This showed that there are sensory deficits in the Het before gross symptomatic onset, consistent with recent studies on human patients. We also suggest that atypical expression of perineuronal nets and dysregulation of the cortical inhibitory network in the MECP2 deficient brain may be a key motif to further understanding RTT pathogenesis.

Perineuronal nets (PNNs) are extracellular matrix structures that form around the soma and proximal dendrites of PV+ GABAergic interneurons, interdigitating with newly formed synaptic contacts after a heightened period of plasticity (Cattaui et al., 2018; Gogolla et al., 2009; Hartig et al., 1992; Miyata and Kitagawa, 2017; Murthy et al., 2019; Sigal et al., 2019). Experiments involving the enzymatic removal of PNN in amygdala, hippocampus, piriform and auditory cortices have shown that synaptic plasticity can be reactivated (Banerjee et al., 2017; Gogolla et al., 2009; Kochlamazashvili et al., 2010; Krishnan et al., 2017; Pizzorusso et al., 2002; Thompson et al., 2018). Mouse models for varying neurological disorders show abnormal/atypical expression of PNNs which, when removed, can greatly improve the associated

pathology or behavioral readouts in these models (Berretta et al., 2015; Krishnan et al., 2017; Pizzo et al., 2016; Reinhard et al., 2019). MECP2 directly occupies the promoter regions of *Gad1* (gene that makes GAD67) and *PV* (Chao et al., 2010; Durand et al., 2012), suggesting that MECP2 controls the activity-dependent expression of these genes. MECP2 regulates many genes (Cohen et al., 2011b; Gabel et al., 2015); therefore, genes encoding for PNN components could also be regulated by MECP2 as they are part of the maturation of the GABAergic circuitry like *PV* and *GAD67*. When PNNs were removed from the SurHet auditory cortex, the SurHets performed more efficiently at the pup gathering paradigm than they had before the PNNs were degraded. However, there was only a partial rescue of the behavior, as they did not perform as efficiently as the SurWT (Krishnan et al., 2017). The partial rescue of behavior suggested that another brain region or circuit may be involved.

The pup gathering behavior is an essential component of maternal care in rodents. This is a multisensory paradigm involving the processing of auditory, tactile, and olfactory cues to find the pups, as well as tactile feedback to identify and gather the pups back to the nest (reviewed in Beach and Jaynes, 1956; Lonstein et al., 2015; Stern, 1996). In first-time dams, tactile feedback from the pups via the perioral area (snout and whiskers) and ventral trunk are essential to learned maternal behaviors like pup gathering (Kenyon et al., 1981; Morgan et al., 1992; Stern, 1990). The pup gathering behavior can also be used as an adult learning paradigm, as Naïve females can learn and efficiently execute this behavior when co-housed with the mother and her pups (Alsina-Llanes et al., 2015; Cohen et al., 2011a; Krishnan et al., 2017). Due to the important role that tactile stimulation plays in pup gathering behavior, we chose to focus on somatosensation as the next sensory motif through which to study MECP2's role in regulating adult experience-dependent plasticity during this complex social behavior.

Tactile sensory input is processed in the primary somatosensory cortex (SS1), a large region of the rodent cortex that is broken into different functional subregions that receive tactile information from specific parts of the body (Brecht, 2017; Feldman and Brecht, 2005; Welker et al., 1992; Xerri et al., 1994, 2005). SS1 also integrates

sensory information from other cortical regions during multisensory behaviors via long-range cortico-cortical connectivity in layers II & III. For example, there is communication between SS1 and the auditory cortex during adult social whisking behaviors, where tactile input to the SS1 modulates the processing of ultrasonic vocalizations in the auditory cortex (Keniston et al., 2010; Rao et al., 2014) as well as communication between the SS1 and motor cortices during social and whisking behaviors (Kinnischtzke et al., 2014; Lee et al., 2013; Mathis et al., 2017; Sreenivasan et al., 2015). The circuitry of this region is well studied but is most well studied in the barrel field cortex (S1BF), which receives tactile information from the whiskers (Filipkowski, 2000; Prigg et al., 2002; Welker et al., 1992). Tactile input from the whiskers reaches layer IV neurons in the S1BF via thalamocortical input from the ventral posteromedial thalamus. This information is then projected to layers II & III or to layers V & VI (reviewed in Aronoff et al., 2010; Chen et al., 2013; Feldmeyer et al., 2013; Petersen, 2007). The deeper layers of the cortex are involved in information output and have been shown to be important for processing tactile information from the whiskers (Lee and Kim, 2012; Park et al., 2020).

Through whole brain manual quantification of PNN density within the SS1, we found that adult Hets had increased PNN density before and after the pup gathering behavior, in a subregion-specific manner (Lau et al., 2020b). NaïveHet PNN density was increased in the barrel field (S1BF) subregion, which receives tactile information from the whiskers, the primary sensory organ mice use to navigate their environment (Arkley et al., 2017; Feldmeyer et al., 2013; Grant et al., 2009; Knott et al., 2002; Welker et al., 1992). As PNNs are markers of restricted plasticity within the brain, we hypothesized that the atypical increase of PNNs within the NaïveHet modifies the animal's tactile perception as well as its ability to learn the pup gathering behavior. However, the maternal paradigm is a complex multisensory behavior, making it hard for us to isolate tactile dysfunction. To test basic somatosensation and whisker-based tactile processing in these mice, we developed a battery of simple tactile tests outlined in the schema in Figure 3.1 (Orefice et al., 2016; Richter et al., 2017; Wu et al., 2013).

We sought to identify if the atypical PNN expression seen in the adult NaïveHet is altering the Het's tactile perception and restricting the animal's ability to perform the pup gathering behavior. Through a time course study, we identified a time point, 6 WO, where the PNN expression does not differ between the WT and the Het in the Naïve state. This suggests that the dysregulatory effect of MECP2 deficiency on PNN formation in the SS1 is delayed, as phenotypic onset of RTT symptoms is delayed in human patients. We found that adult 12 WO Hets have altered tactile perception when compared to WTs whereas 6 WO Hets do not show deficits in tactile perception when compared to age-matched WTs. This result suggests that the atypical increase of PNNs within the 12 WO NaïveHet SS1 is causing altered tactile perception in these animals. After completing the simple tactile behaviors, the 6 WO presents with subtle alterations in SS1 PNN density when compared to the WT. Despite not showing deficits in tactile perception, we report that 6 WO SurHets are inefficient at the pup gathering behavior and have significantly higher PNN densities within the SS1 and its subregions compared to the SurWT and NaïveHet. Taken together, this suggests that MECP2 influences SS1 PNN expression in complex, but not simple, contexts within the 6 WO age group.

Methods

Animals

All experiments performed on female mice of either 12, 10, 8 or 6 weeks of age between the hours of 9 am and 6 pm (during light cycle). All animals raised on a 12/12-hour light/dark cycle and received food ad libitum. Environmental enrichment (paper cones and 2 nestlets) was not changed during the raising of these animals as to not distort the studies due to new or novel objects being put in the cage. Animals used were CBA/CaJ (maternal behavior), *Mecp2^{Het}* (C57BL/6, B6.129P2(C)-*Mecp2^{tm1.1Bird}*/J) and *Mecp2^{WT}*. All procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and approved by the University of Tennessee-Knoxville Institutional Animal Care and Use Committee.

Simple Tactile Tasks Assay:

Behavioral Setup:

Subjects went through a six-day battery of behaviors to test tactile processing through the whiskers as represented in Figure 3.1 (all figures in appendix at the end of chapter). Subjects were cohoused with littermates leading up to this task (1-3 housemates), no manipulations were made to the home cage once the assay had begun. The behavior box was a plexiglass material, 40 cm³. Two sets of holes were drilled in the bottom of the box for the texture/object inserts. They were spaced so that the textures and objects were 4 cm apart. Holes that were not covered by the insert during the behavior had tape placed over them so the subject would not fall through, this is how the holes were covered during the habituation periods. The chamber was placed in a sound-proof box and behavior was recorded from the top and bottom of the set up. The behavior chamber and inserts were cleaned with MB-10 and allowed to dry completely before the start of each behavior day and between each animal to eliminate scent bias.

Texture discrimination inserts were 0.5 cm x 4 cm x 15 cm and made of plexiglass so the sandpaper textures could slide on and off the inserts to be cleaned. The textures are made of 3M sandpaper of varying grit; a course 80 grit (190 µm), a medium 120 grit (115 µm) and a fine 220 grit (70 µm). Rodents can differentiate between textures that are as close as 25 µm to one another (Wu et al., 2013), however in literature researchers often use larger differences in texture, such as using a textured object and a smooth object when testing for texture discrimination in both head fixed and free moving studies (von Heimendahl et al., 2007; Orefice et al., 2016; Prigg et al., 2002). We elected to test two texture differences in the event that our mice would be able to differentiate between a larger difference but not a smaller difference in texture, similar to (Wu et al., 2013). Novel object recognition inserts were cubes and spheres made of plexiglass and had the same dimensions (4 cm x 4 cm x 4 cm) (Orefice et al., 2016). Adhesives for the adhesive test were 1cm (Fleming, 2004; Richter et al., 2017).

Behavioral Paradigm: Outlined in Figure 3.1

Habituation: On days 1 and 2 of behavior, the mouse was placed into the behavior chamber for 10 minutes in order to familiarize them with the new environment.

Texture Discrimination/Novel Object Recognition: On days 3-5, the mice went through a texture discrimination and novel object recognition assay. In general, this starts with a *familiarization* phase, where the mouse is placed into the behavior chamber with two identical textures/objects and is allowed to investigate for 10 minutes. The mouse is then removed and placed back into the home cage for a 5 minute '*retention* period'. During the *retention* period, both textures/objects are removed from the behavior chamber and replaced with two new textures/objects, one identical to that from the *familiarization* phase and the new novel texture/object. At the end of the 5 minutes, the mouse is then placed back in the behavior box and is filmed investigating the textures for another 10 minutes (*testing* phase). For texture discrimination, animals were familiarized to identical textures (course sandpaper) and the novel textures differed on day 3 and day 4; medium and fine sandpaper, respectively. For novel object recognition (day 5), the mice were familiarized to plexiglass cubes and the novel object was a plexiglass sphere.

Adhesive Test: On day 6, the mice were run through three trials of the adhesive test. For this test, a sticker was placed on the head of the mouse between the eyes and the mouse was recorded until the sticker was removed or three minutes had elapsed, whichever came first. The mouse was given a three-minute break between each trial (Fleming, 2004; Richter et al., 2017).

Analysis:

Texture Discrimination and Novel Object Recognition: All behaviors were manually scored using Datavyu software (Datavyu Team, 2014). All analyzers were blinded to the age and genotype of the mice during analysis. The videos were scored for the time intervals in which the mouse investigated the novel versus the familiar object throughout the 10-minute testing phase, in seconds. Investigative interactions were then coded as...

Whisker (w) : whisker and nose investigation

Approach (a) : the head being within 3 cm of the object

Paw (p) : investigation with the forepaws

Climbing (c) : all four paws off the ground and touching the object

All approaches were classified as whisker interactions. These classifications and qualifications were taken from primary literature and are commonly used when scoring these assays (Orefice et al., 2016; Pacchiarini et al., 2017; Wu et al., 2013). However, we classified approach as the mouse being within 3 cm of the object rather than the traditional 2 cm, due to the average whisker length of an adult mouse being around 3.7 cm (Wright, 1965). Mice were excluded from the analysis if they did not interact with one or both objects or had an investigation time under 2 seconds during the testing phase (Orefice et al., 2016; Patrick Wu and Dyck, 2018). Numbers of animals included in the analysis for each behavioral day and number of animals excluded for those days are outlined in Supplemental Figure 3.1.

Traditionally in texture discrimination assays paw and climbing interactions are excluded from the analysis to focus on whisking (Wu et al., 2013). However, texture discrimination by forepaw interaction has also been tested, by eliminating whisker interactions by removing whiskers (Orefice et al., 2016) or placing the textures on the floor of the cage (Xerri et al., 2005). For our analysis, we were able to separate the investigation times into whisker, forepaw and climbing interactions for each animal. Our results are reported as raw investigation times, split by interaction type, as well as the novelty recognition index, which normalizes exploration time differences between animals and is widely used in the current literature (Antunes and Biala, 2012; Pacchiarini et al., 2017; Wu et al., 2013). Novelty recognition index is the percentage of investigation time spent with the novel object/texture divided by the total investigation time, normalizing the differences in exploration time between animals; values over 50% shows novelty preference.

Adhesive Test: All behaviors were manually scored by analyzers that were blinded to the age and genotype of the mouse. For each trial, the experimenter recorded the time it took for the mouse to first attempt to remove the adhesive (latency to first attempt), the number of failed attempts to remove the adhesive (errors), the time at which the adhesive was successfully removed (latency to removal), as well as the time between the first attempt and the successful removal during which all the errors would have occurred. The performance in each category over the three trials was

averaged together to get the score for the mouse. Statistical analysis and graphs were generated using GraphPad Prism.

Pup gathering assay:

Behavioral Setup:

The pup gathering behavior was performed similarly to Krishnan et al., 2017. Two Naive females, one *Mecp2^{WT}* and one *Mecp2^{Het}*, are co-housed with a pregnant CBA/CaJ female 3-5 days before birth; these Naive females are then termed 'surrogate'. Once the pups are born, the gathering behavior is conducted on post-natal day 0 (P0), P3 and P5 for each adult separately. First, the adult is placed in the home cage with the pups for 5 minutes (*habituation*) before the pups are removed for 2 minutes (*isolation*). The pups are then placed back into the cage, scattered into the corners and the center of the cage (*retrieval*) (Figure 3.6 A). The adult was then recorded for 5 minutes, if all pups were not retrieved to the nest by the 5-minute mark, they were placed back in the nest in preparation for the next adult. After the behavior was completed, all mice were returned to the home cage. These were performed in a sound-proof dark box during the light cycle (between 9 AM and 6 PM). Surrogates were perfused immediately after P5 behavior. There were 6 behavioral cohorts.

Analysis:

All videos were assigned a number to blind the experimenter to the identity of the mouse. Each video was manually coded for latency, the amount of time required to retrieve all of the pups back to the nest (with a max time of 5 minutes) and errors, the number of interactions with the pups that did not result in a successful retrieval. Latency was calculated as:

$$Latency\ index = [(t_1 - t_0) + (t_2 - t_0) + (t_n - t_0)] / (n \times L)$$

Where n = number of pups outside the nest, t_0 = start of trial, t_n = time of the pups successful retrieval to the nest, L = trial length. Statistical analysis and graphs were generated using GraphPad Prism.

Immunohistochemistry

All the animals were perfused with a 4% paraformaldehyde/PBS solution either after behavior or when they became the correct age (time course study).

The brains were extracted and stored in 4% paraformaldehyde/PBS solution overnight, which was exchanged for PBS for storage until processing. Before sectioning, samples were incubated overnight in a 30% sucrose/PBS solution at room temperature. Brains were sectioned at 100 μ m in a coronal orientation using a freezing microtome. The left hemisphere was denoted by a small cut or hole in ventral part of the section. Free-floating sections were immunostained as described in Lau et al., 2020b. Briefly, sections were blocked using a 10% normal goat serum and 0.5% Triton X-100 solution at room temperature (RT) for 3 hours before an overnight primary incubation step with biotin-conjugated WFA lectin (1:500; Sigma-Aldrich) and rabbit MECP2 primary antibody (1:1000; Cell Signaling) in a 5% normal goat serum and 0.25% Triton X-100 solution. The next day, the sections were incubated in a secondary solution (5% normal goat serum and 0.25% Triton X-100) containing Alexa Fluor 488 and Texas-Red secondary antibodies (1:1000; Invitrogen) for 4 hours. Sections were then counterstained with the nuclear marker, DAPI (1:1000) for 5 minutes. The free-floating sections were mounted onto slides using Fluoromount-G (Southern Biotech).

Time course cohorts were stained in age matched pairs (i.e., one 8 WO Het and one 8 WO WT). Pup gathering cohorts containing a NW, NH, SW and SH were stained together. Simple tactile cohorts were stained in pairs as well, a TacWT and TacHet.

Microscopy and Image Analysis

PNN: Whole brain PNN epifluorescent images were acquired using a Keyence semi-automized microscope (Keyence BZ-X710; Keyence Corp.). Each hemisphere of the section was imaged at 10x in the single-plane and stitched using BZ-X Analyzer (Keyence). Imaging settings for each cohort was based on the *Mecp2*^{Het}; for time course it was the Het, for pup gathering behavior SH, and simple tactile TacHet. To reduce overexposure, the exposure time was set by finding the point at which the first saturated pixel appeared in the sample, then reducing the exposure by one unit (based on the software). This was done for all Het sections, including both hemispheres, then most common lowest exposure time was used to image all sections within that cohort.

High-intensity PNN quantification was done using ImageJ software (Schneider et al., 2012). The stitched image was opened in ImageJ, then the corresponding

coronal map template from Paxinos and Franklin's *The Mouse Brain* (Paxinos and Franklin, 2013) atlas was overlaid onto the micrograph. The SS1 and its subregions were outlined based on the map, during which they were separated into 'top layers' containing layers I-IV and 'bottom layers' containing layers V-VI. The lower boundary of the cortex was indicated by the top of the corpus collosum (in the micrograph). High-intensity PNNs were quantified by maxing out the contrast of the image and manually marking the signals that were 80% of the original PNN and higher, as described in Lau et al., 2020b and Layo et al., 2020. PNN density was calculated dividing the total # of high-intensity PNNs by the area of that region; PNN density for layers were calculated similarly. Statistical analysis and graphs were generated using GraphPad Prism.

MECP2: All images for determining the percentage of cells expressing MECP2 in the SurHet SS1 were taken on a Leica SP8 confocal microscope with a 40X oil immersion objective X 2 zoom. All z-series images were 25-30 μm thick and covered a 145.31 μm^2 of the left S1BF; four z-stacks were taken per animal (n = 6 animals). For each z-series the number of DAPI+ and MECP2+ cells were quantified separately using the FIJI plugin 3D Cell Counter (Bolte and Cordelières, 2006; Schindelin et al., 2012). Percentage of MECP2+ cells within an image was calculated by dividing the number of MECP2+ cells by the number of DAPI+ cells. Each data point in Figure 3.6 D & G represents the average percentage value from four z-series per animal (color coded to highlight individual variability). Supplementary Figure 3.3 shows the variation per image for each individual animal.

Results

Adult Hets show atypical tactile somatosensation

Previously, we found that the atypical increase in PNN density within the NaïveHet was present in the primary somatosensory barrel field (S1BF) subregion, which receives tactile input from the whiskers (Lau et al., 2020b). We developed a series of simple tactile tasks to test if whisker-based tactile perception or basic somatosensation were altered in the 12 WO Het mice (Figure 3.1). Briefly, the mice are habituated to the behavior box during the first two days. This is followed by two days of texture discrimination assays, where the mice use their whiskers to investigate

the textures presented to them (Orefice et al., 2016; Wu et al., 2013). On day 5, they go through a novel object recognition test, which is the same premise as the texture discrimination test, but these objects differ in shape, not texture (Ennaceur and Delacour, 1988). The purpose of both texture discrimination and novel object recognition tasks, is to stimulate the mouse's innate curiosity and to measure the duration of time and mode through which the mouse investigates the textures/objects (Ennaceur, 2010; Şık et al., 2003). On the last day of behavior, the mice undergo the adhesive test, which is meant to test basic somatosensation (Fleming, 2004). Animals that have gone through the simple tactile assays are termed TacHet or TacWT.

The texture discrimination and novel object recognition tasks were picked specifically because both have been widely used in testing whisker-based somatosensation. Whisker stimulation, whether passive or active, induces structural plasticity changes within the S1BF of adult mice (Knott et al., 2002; Kuhlman et al., 2014; Prigg et al., 2002; Welker et al., 1992). Many early studies on whisker involvement in texture discrimination or 'roughness detection' were conducted by plucking or trimming the whiskers, to remove those sensory organs, and discovering that the whiskerless mice were unable to discriminate between the textures (Guic-Robles et al., 1989; Lipp and Van der Loos, 1991). These studies then ablated the S1BF region of the cortex and discovered that the barrelless animals were also unable to discriminate between textures, suggesting that the S1BF was an important brain region for this behavior (Guic-Robles et al., 1992). More recent studies have shown that the processing of tactile input in the S1BF is critical for texture discrimination (Grant et al., 2009; Helmchen et al., 2018; Park et al., 2020; Wiest et al., 2010) and object recognition tasks (O'Connor et al., 2010; Rodgers et al., 2021).

Texture discrimination and object recognition tasks are typically scored on a novelty preference scale, which may be reported as a percentage or ratio of time spent with the novel texture/object compared to the familiar texture/object (Ennaceur and Delacour, 1988; Lipp and Van der Loos, 1991). In the 10-minute testing phase for all three tasks, we collectively do not see novelty preference in the 12 WO TacWT or TacHet animals (Figure 3.2 A, D, G). However, we see that some TacWTs and

TacHets do show novelty preference with percentage values closer to 75%. The novel object recognition task and texture discrimination assays have been widely used and there is no general consensus on the 'correct' time parameters to use for the familiarization, retention, and testing phases (reviewed in Antunes and Biala, 2012). Abdelkader Ennaceur, whose 1988 publication kicked off the novel object recognition tasks popularity, revisited the assay in a 2010 review where he stated that timing is particularly important as the novelty of an object or texture can be short lived (Ennaceur, 2010; Ennaceur and Delacour, 1988). In a 2005 study on rats, Ennaceur found that novelty preference began to decrease after 3 minutes of the behavior (Ennaceur et al., 2005). Many studies use the first 3 minutes of the testing phase for analysis of novelty preference (Dellu et al., 1992; Dix and Aggleton, 1999; Ennaceur et al., 2005; Wu et al., 2013). When we analyzed the first three minutes of the texture discrimination day 1 recordings, we saw that the 12 WO TacWT showed a statistically significant novelty preference where the TacHets did not (Supplementary Figure 3.1 A). This however did not work for texture discrimination day 2 because only three TacWT interacted with the textures before the 3-minute mark (data not shown).

When compiling the investigation data for texture discrimination, we noticed that there were large disparities in exploration time and type between the two genotypes. In both texture discrimination trials, we show that TacHet spend significantly more time exploring the textures when compared to WT, though there is no difference between investigation times of the novel and familiar textures within genotype (Figure 3.2, B & E). Here we include all interactions, whisker, forepaw and climbing, however most current literature only looks at one of these factors, depending on the purpose of their study; whisking tactile perception deficits (Wu et al., 2013) or peripheral somatosensation deficits (Orefice et al., 2016), for example. The 12 WO TacWTs exclusively use their whiskers to investigate the textures (purple, Figure 3.2, C & F). In contrast, TacHets rely on both their whiskers, forepaws, and body (climbing) to interact with the textures (green, Figure 3.2, C & F). Note, no climbing interactions are seen in the first three minutes of the behavior, therefore are not in the 3-minute analysis (Supplementary Figure 3.1). This result matches reports from studying whiskerless

mice, who will compensate for the loss of sensory input from the whiskers by using their forepaws to investigate the textures (Orefice et al., 2016; Wu et al., 2013; Xerri et al., 2005). This also goes back to one of the earlier studies on texture discrimination in the barrel cortex. Animals that were able to discriminate between textures lost the ability to do so after S1BF lesioning. When these same animals were allowed to investigate the textures with their forepaws, they were able discriminate between textures again (Guic-Robles et al., 1992). 12 WO TacHets show this compensatory investigation as they use both their whiskers and body to investigate the textures, whereas the 12 WO TacWTs do not.

We see no behavioral differences between the TacWT and TacHets in the object recognition assay or adhesive test. The TacHets have similar exploration times when compared to the TacWT and both conditions use a mix of whisker, paw, and body interactions when investigating the objects (Figure 3.2, H & I). One reason for this, may be because the mice are easily able to climb on the objects. We also note that most paw interactions in this assay precede climbing interactions. From the adhesive test, we show that there is no difference in basic (not whisker-related) somatosensation between the TacWT and TacHet (Figure 3.3). There is no significant difference in the average amount of time it takes for the mouse to attempt to remove the adhesive (B), the time to successful removal (A), or the number of removal attempts made during the three trials (C) (Figure 3.3). These results suggest that the 12 WO TacHet has altered tactile perception, specifically during simple whisking behaviors, which may be caused by the atypical increase in PNN density in the 12 WO NaïveHet SS1.

6 WO NaïveHet show no increased PNN density compared to WT

In order to identify if the atypical increase in PNN density within the 12 WO NaïveHet was altering tactile perception in TacHet as well as altering pup gathering efficiency in SurHets, we performed a time course to identify a time point at which there was no difference in PNN density between the NaïveWT or NaïveHet SS1. Through whole SS1 quantification of PNN expression of NaïveWT and NaïveHet brains at 12 WO (the oldest animal in the previous 2020 study), 10 WO, 8 WO, and 6 WO; we found

that there was no difference between the 6 WO NaïveWT and NaïveHet SS1. Table 3.1 represents the average PNN density within the SS1 as well as S1BF, S1ULp and S1FL subregions (n = 3 animals per genotype per age). Both 12 and 10 WO Hets have significantly higher PNN density in the whole SS1 as well as S1BF. Additionally, PNN density is increased in the S1ULp of the 12 WO Het. There is no significant PNN density increase within the whole SS1 of either the 8 or 6 WO Het. However, there is a significant PNN density increase within the S1FL subregion of the 8 WO Het. At 6 WO, there is no significant difference in PNN density between the NaïveWT or NaïveHet within the SS1 or its subregions, suggesting that the Het at this age would be able to perform the simple tactile tasks and pup gathering behavior comparably to the WT.

6 WO TacHets do not show tactile perception deficits

We repeated the simple tactile assays with 6 WO Het and WT females to test if it was the atypical increase of PNN density in the SS1 of the 12 WO Het that was contributing to altered tactile perception. We did not find significant novelty recognition in the 6 WO TacWT or TacHet on texture discrimination days 1 and 2 or in the object recognition assay (Figure 3.4 A, D, G). However, on texture day 1 we saw a trend that most of the TacWTs had a novelty percentage below 50%. This indicates a familiarity preference (Ennaceur et al., 2005), showing that individual TacWT can discriminate between the textures, they just prefer the familiar texture to the novel one (Figure 3.4 A). On texture discrimination day 2, we see that many of the TacHet show novelty preference, though it is not significant as a whole group via one sample t test at 50% chance, while TacWT is evenly split between novelty and familiarity preference (Figure 3.4 D). Most animals hover between 40-60% (no preference) in the object recognition task (Figure 3.4 G).

We found that there was no difference in total investigation time between the 6 WO TacWT or TacHet on across all the behaviors (Figure 3.4, B, E, H). When the interaction categories are split, both the TacWT and TacHet rely mostly on their whiskers to investigate the textures on texture discrimination days 1 and 2. TacHet uses exclusively the whiskers to interact with the textures on both days (Figure 3.4, C

& F), in contrast to the 12 WO TacHet (Figure 3.2, C & F). The same can be said for TacWT, other than a single outlier animal that performed similarly on both texture discrimination assay days (purple circle with x thru it) and another animal on the second day of the behavior (purple diamond), who were littermates and cage mates during the behavior (Figure 3.4 C & F). Like the texture discrimination data, we found no differences in behavior during the object recognition task between the TacWT and TacHet (Figure 3.4, H & I). We found no difference in basic (not whisker-related) somatosensation between the 6 WO TacWT and TacHet in the adhesive test (Figure 3.5). In contrast to the 12 WO TacHet and TacWT data, these data suggest that the 6 WO Het does not have altered tactile perception when compared to the WT.

6 WO Surrogate Hets are inefficient at pup gathering

Next, we wanted to test whether the 6 WO Het would be able to efficiently perform the pup gathering behavior without this atypical increase in PNN density within the SS1 before going through the behavior. In this paradigm, as outlined in Figure 3.6 A, two 6 WO Naïve females, NaïveWT and NaïveHet, are co-housed with a first-time pregnant female. Once co-housing occurs, the status of the Naïve females changes to Surrogate (Sur) indicating the change in experience that comes with exposure to this paradigm. The pup gathering paradigm was conducted on the day the pups were born, post-natal day 0 (P0), then on P3 and P5. For the assay, the pups were scattered around the home cage before an adult was placed into the cage. We measured efficacy of retrieval based on latency, how long it takes the adult to retrieve the pups back to the nest, and errors, the number of interactions between the adult and pup that result in an unsuccessful retrieval. This was repeated with the mother and each surrogate on the three days.

The 6 WO SurWT perform the pup gathering behavior efficiently (latency, Figure 3.6 B & C) and accurately (errors, Figure 3.6 E & F). As an average over the three days of behavior, there is little individual variability in the SurWT performance over latency and errors (Figure 3.6 B & E). As a whole, the SurWT improve throughout the behavioral paradigm from P0 to P5 in both efficacy measurements (Figure 3.6 C & F).

While 6 WO SurWT is efficient in this paradigm, 6 WO SurHet is not. As an average over the three behavior days, SurHet have significantly more unsuccessful retrieval attempts and take longer to retrieve the pups back to the nest than their WT counterparts (Figure 3.6 B & E). This trend is also seen in the average performance of SurHet on each day of the paradigm, where they consistently take longer to retrieve pups back to the nest when compared to SurWT (Figure 3.6 C). On P5, there seems to be a slight reduction in the average amount of errors, however it is not a statistically significant improvement like what was seen in the WT (Figure 3.6 F).

Mosaic expression of MECP2 has been correlated to phenotypic variability within RTT human patients and the RTT mouse model (Krishnan et al., 2017; Rietveld et al., 2015; Xinhua Bao et al., 2008; reviewed in Ribeiro and MacDonald, 2020). We report a negative correlation between the percentage of MECP2+ cells and the average number of errors performed by the SurHet (Figure 3.6 G). A similar trend was seen with normalized latency (Figure 3.6 D), though neither correlation is statistically significant.

Though there is no atypical difference in PNN expression between the 6 WO NaïveWT and NaïveHet, as there was in the adult animals, and no atypical performance in the simple tactile behavior assays, the 6 WO SurHet is inefficient at the pup gathering behavior when compared to the age matched SurWT. Based on previous work in the adult SurHet, this data suggests that experience-dependent plasticity changes may be dysregulated within the SS1 in response to the pup gathering behavior (Krishnan et al., 2017; Lau et al., 2020b).

6 WO SurHet have increased PNN density within the SS1

To investigate experience-dependent plasticity changes in the SS1, we quantified high-intensity perineuronal net (PNN) expression throughout the SS1 based on the Paxinos and Franklin's Mouse Brain atlas (Paxinos and Franklin, 2013). A benefit to this form of analysis is the finer subregion-specific resolution that encompasses the whole rostral to caudal axis of the SS1 (~ 3.5 mm). In the whole SS1, there is a significant increase in PNN density within the SurHet when compared to its Naïve counterpart as well as the SurWT, with individual variability between

animals within condition (Figure 3.7, E). These trends are pervasive in the left and right hemisphere of the SS1 (Figure 3.7, F).

During analysis, quantification of high-intensity PNN expression was separated into ‘top’ and ‘bottom’ layers. The ‘top’ layers contained layers I-IV, which are sensory input and cortico-cortical communication layers in the SS1 (Feldmeyer et al., 2018). The bottom layers contain layers V-VI, which are sensory output layers (Petersen, 2007). Alterations in PNN density that are occurring in either layer grouping or in both could be indicative of circuitry involved in this behavior. We found that the top layer has higher high-intensity PNN density than that of the bottom layers across all conditions in the SS1 (Figure 3.7, G). When comparing between conditions within layer grouping, the SurHet maintains a higher PNN density when compared to SurWT and NaïveHet (Figure 3.7, G). The difference between conditions in both whole and layer high-intensity PNN expression is shown in Figure 3.7 A-D.

Alterations in PNN density across conditions are subregion- and hemisphere-specific

The SS1 is composed of multiple functional subregions that integrate tactile information that comes from specific regions of the body. We focused on subregions that receive tactile information from the face (upper lip, S1ULp) and whiskers (barrel field, S1BF) as the adults use their whiskers to navigate their environment and their mouth to pick up pups (Figure 3.8, A & B). Sensory input from the perioral area (snout and whiskers) are important for proper execution of the pup gathering behavior (Kenyon et al., 1981; Morgan et al., 1992). We also quantified high-intensity PNN expression within the S1FL, which receives tactile information from the forelimb/paws, and S1DZ, the dysgranular zone, which is thought to be involved in proprioception (Chapin and Lin, 1984); both regions were shown to have alterations in PNN density during the pup gathering behavior in the adult surrogates (Lau et al., 2020b) (Figure 3.8, C & D). We also quantified PNN density within these subregions by top and bottom layers, which can help indicate a more specific circuit that is being used in the WT and Hets in response to this behavior. Similar to the overall SS1 PNN density layer measurements, the top layers (Table 3.2) of each subregion have significantly higher

PNN densities than the corresponding bottom layer (Table 3.3) across all conditions and subregions.

In all of the subregions, the SurHet has a significantly higher PNN density when compared to the SurWT, when looking at the whole region (Figure 3.8, A-D). Additionally, SurHet has significantly higher PNN density than the NaïveHet in both the S1BF and S1DZ (Figure 3.8, A & C), but not in the S1FL or S1ULp subregions (Figure 3.8, B & D). Each subregion is contributing to the increase in PNN density seen in the overall SS1 of the SurHet (Figure 3.7, E). Significant changes in PNN density within the top layers and bottom layers across conditions remain the same in the S1BF as they were in the combined dataset (row 3, Table 3.2 & 3.3). However, in the S1ULp SurHet has a higher PNN density compared to SurWT only in the bottom layers (row 4, Table 3.3).

We analyzed these subregions in a way that we can split the hemispheres within each region (Figure 3.8 A' & B'), which is also done within the layer analysis mentioned before (Tables 3.2 & 3.3; summarized in Figure 3.12 A & B). Some subregions, undergo hemisphere- and layer-specific changes in PNN density while others model what is seen in the combined hemisphere data. We also see that individual variability between cohorts is consistent across subregions but is more obvious when the hemispheres are split. The S1BF is one of the regions that does not show unique hemisphere- or layer-specific changes in PNN density. Within this region, SurHet has significantly higher PNN densities than NaïveHet and SurWT, which is pervasive in the left and right hemisphere (Figure 3.8, A'), as well as the top and bottom layers (row 3, Table 3.2 & 3.3). In contrast, the S1ULp undergoes hemisphere- and layer-specific changes in PNN density. There is a significant increase in PNN density in the SurHet compared to SurWT, which is isolated to the right hemisphere (Figure 3.8 B') and seen in the top and bottom layers (row 4, Table 3.2 & 3.3). Additionally, there is a significant increase in PNN density within the top layer of the NaïveHet compared to the NaïveWT in the left hemisphere (row 4, Table 3.2).

Within the S1DZ, the significant increase in PNN density in the SurHet compared to NaïveHet and SurWT is isolated to the left hemisphere (Figure 3.8, C').

There is an asymmetrical increase in PNN density within the left hemisphere when compared to the right hemisphere of the SurHet S1DZ (Figure 3.8, C'). Both trends in PNN density are present within the top layers (row 6, Table 3.2). However, SurHet has significantly higher PNN density only when compared to NaïveHet within the bottom layers of the left hemisphere in the S1DZ (row 6, Table 3.3). In contrast to the other subregions, the significant increase in PNN density of the SurHet compared to the SurWT within the S1FL does not hold up in either hemisphere (Figure 3.8, D-D'), but is present in the bottom layers, only in the left hemisphere (row 5, Table 3.3). Instead, we found that there is an increase in PNN density within the right hemisphere of the NaïveWT compared to the left hemisphere, which is also present in the top layers (Fig 3.8, D'; row 5, Table 3.2). Within the left hemisphere, there is a significant increase in PNN density within the Het after behavior, which is seen in the top layers as well (Fig 3.8, D'; Table 3.2). Within the right hemisphere, there is also an increase in PNN density seen after the pup gathering behavior, but this time in the WT (Figure 3.8, D'). This change in the WT is present in both the top and bottom layers (Table 3.2 & 3.3).

These subregion-specific changes in PNN density that are occurring within the SS1 after the pup gathering behavior suggest that 1) there are normal plasticity changes, as marked by PNN expression, that occur in the WT in response to this behavior and 2) PNN expression is being dysregulated in the MECP2 deficient mice during the complex multisensory behavior.

6 *WO SurHet have reduced SS1 areas within sub-regions*

We found that there was a reduction in area of the S1ULp, S1DZ and S1BF subregions in the SurHet compared to the SurWT (Figure 3.9, A-C) as well as when compared to the NaïveHet (Figure 3.9, A & C). While there was variability in PNN density between individual animals within condition (Figure 3.8), that is not seen in the area measurements of any subregions (Figure 3.9). There was no change in S1FL area (Figure 3.9, D). The SS1 is a highly plastic region of the brain and has been shown to change in size in response to stimuli (Feldman and Brecht, 2005; Knott et al., 2002; Petersen, 2007; Posluszny et al., 2015). In the *Mecp2*-null male model, there is an increase in inhibitory signaling (Dani et al., 2005; Lee et al., 2017) which is

accompanied by morphological reductions in dendritic branching, neuron size and overall S1BF area (Lee et al., 2017; Moroto et al., 2013; Smith et al., 2019). Though these studies have not been done in the Het S1BF, we do know that MECP2- neurons in the Het brain (motor cortex) resemble those from the null brain in morphology (Rietveld et al., 2015; reviewed in Ribeiro and MacDonald, 2020). The mosaic expression of MECP2 in the Het brain could be causing increased inhibition within the S1BF in response to this behavior, reducing signaling in the region and, in turn, the area. This speculation matches with the increased PNN density in the SurHet, which is indicative of a matured inhibitory network. Together, this suggests that the increase in PNN density within the SurHet maybe restricting plasticity within the region, resulting in less activity within these subregions as shown by the reduced areas.

6 WO TacHets present with very subtle increase in PNN density in SS1 after simple tactile paradigm

As 6 WO TacHets do not present with deficits in tactile perception (an asocial behavior) but do perform poorly in the pup gathering behavior (a complex social behavior) we wanted to see if PNN expression changes were a result of the complex social paradigm or a result of the 6 WO Het going through any new tactile related experience. In the overall SS1, we found that the 6 WO TacHet has a slightly increased PNN density when compared with the TacWT (Figure 3.10, A). However, when the hemispheres were split, this significant difference is no longer present (Figure 3.10, B). During the analysis of these sections, we noticed that the significant difference in PNN density was being driven by one cohort, represented by the peach-colored circle (Figure 3.10, A & B). As the 6 WO Tac PNN analysis is based on four cohorts (n = 4 per genotype), we expect the subtle differences in PNN density between TacHet and TacWT to not be present with the addition of two more cohorts, making the PNN density analysis n = 6 per genotype, like the pup gathering cohort.

We found that the top layers of the SS1 contained significantly more PNNs than the bottom layers, similar to the pup gathering dataset (Figure 3.10, D). In contrast to the pup gathering dataset (Figure 3.7 G), the significant changes in PNN density found in the whole SS1 (Figure 3.10, A), are not present when the layers are split (Figure

3.10, D). There are no area differences in the SS1, or any of its subregions, between the two genotypes (Figure 3.10, C). When compared to the 6 WO pup gathering data, these findings suggest that MECP2 deficiency is causing the dysregulation of PNN formation within the SS1 in a context-dependent manner.

6 WO TacHet PNN density increases are isolated to specific subregions, but do not occur in a hemisphere- or layer-specific manner

We found that subtle PNN density increases within the 6 WO TacHet occur within the whole S1BF, S1FL and S1DZ (Figure 3.11 A; Table 3.4). This is present in both the top and bottom layers for S1BF and S1FL (Table 3.5 & 3.6). When the S1DZ is split into top and bottom layers, the significant increase in TacHet PNN density is no longer present (Table 3.5 & 3.6). Like the whole SS1, when the subregions are split into left and right hemisphere any significant difference between the TacHet and TacWT is no longer present (Figure 3.11 A'; Table 3.4). This holds true in both layers as well (Table 3.5 & 3.6; summarized in Figure 3.12 C & D).

We found no significant differences in PNN density between the TacHet and TacWT within the S1ULp, S1J (jaw), S1HL (hindlimb), S1Tr (trunk) or S1Sh (shoulder) when looking at the whole subregion or individual hemispheres (Figure 3.11 B, B', Table 3.4). Other than a significant increase in PNNs in the bottom layer of the S1ULp, we found no significant changes in PNN density within the top or bottom layers for these regions (Table 3.5 & 3.6; summarized in Figure 3.12 C & D). These findings further support our speculation that MECP2's involvement in experience-dependent plasticity, at the 6 WO time point, is dependent on the complexity of the behavioral contexts.

Discussion

An emerging hypothesis in RTT pathogenesis is that subtle deficits in sensory processing, precede the onset of higher-order cognitive deficits and phenotypes that are characteristic of the disorder. Here, we identify distinct behavioral and cellular phenotypes in the *Mecp2*-Het female mouse model in response to complex and simple sensory behaviors. We show that MECP2 deficiency affects SS1 experience-

dependent plasticity, as shown through the formation of perineuronal nets (PNNs), in a context-dependent manner. Mature PNNs are thought to be inhibitory to synaptic plasticity, as they form around PV+ interneurons within the cortex after an experience (Hartig et al., 1992; Hou et al., 2017; Pizzorusso et al., 2002; Ueno et al., 2018). For example, during the visual critical period, visual experience induces increased expression of inhibitory markers, such as GAD67, PV, and PNNs all of which mark the termination of the critical period (Beurdeley et al., 2012; Hou et al., 2017). When these PNN structures are enzymatically removed from the mature, adult visual cortex plasticity is restored to the region (as measured by ocular dominance) (Pizzorusso et al., 2002, 2002).

Previously, we showed that the maturation of the visual cortex was accelerated in the *Mecp2-null* male, as measured by precocious expression of these inhibitory markers (Krishnan et al., 2015). Similarly, dysregulation of the inhibitory network was seen in the adult *Mecp2-Het* auditory cortex after the multisensory pup gathering paradigm, resulting in inefficient pup retrieval (Krishnan et al., 2017; Lau et al., 2020a). When PNNs were enzymatically removed from the auditory cortex, there was a partial rescue of the retrieval behavior indicating that another region is involved in this behavior (Krishnan et al., 2017). The SS1 showed alterations in PNN expression within the adult *Mecp2-Het* brain, suggesting that this region is also undergoing maladaptive alterations in the inhibitory network as a result of MECP2 deficiency (Lau et al., 2020b). Within SS1, *Mecp2-null* males studies show that inhibitory signaling is increased (specifically barrel field) of these animals in comparison to WT (Dani et al., 2005; Lee et al., 2017). While these studies have not been done in *Mecp2-Het* females, they do support that MECP2 deficiency causes a shift in the inhibitory network of this region which may become exacerbated when going through multisensory experience-dependent events. Here we contribute two new contexts in which the regulation of experience-dependent plasticity by MECP2 can be studied.

Are there tactile-based sensory processing deficits in the adult Het?

In Lau et al 2020, we showed that adult Hets had an increase in PNN expression in the SS1 compared to adult WTs before going through the pup gathering behavior,

which adult Hets perform inefficiently at (Lau et al., 2020b). As somatosensation is a primary sense mice use to interact with their environment and plays an important role in efficient pup gathering (Kenyon et al., 1981; Morgan et al., 1992; Xerri et al., 1994), this finding suggested there may be tactile-based sensory processing deficits in the adult Het caused by this atypical increase of PNNs in the SS1.

In order to test tactile perception and sensation in a simple context, we developed a series of simple tactile tasks to test basic somatosensation and tactile perception through the whiskers (Figure 3.1). Analysis based on categorizing the way that the mice interact with the textures in the texture discrimination assay showed that 12 WO TacHets use their whiskers, forepaws, and bodies to investigate the textures, whereas the TacWT only use their whiskers. The different interaction types used by the TacHet, indicate that they are compensating for a lack of tactile feedback through the whiskers, by investigating with other parts of their bodies, similar to whiskerless mice. Whiskerless mice have had their whiskers either trimmed or plucked, so the S1BF is receiving no tactile input (Guic-Robles et al., 1989). A similar result is seen in barrelless (by lesioning) mice, as they are not able to integrate information from the whiskers in the cortex but compensate for a lack of tactile input in the S1BF by using their forepaws to investigate the textures (Guic-Robles et al., 1992; Orefice et al., 2016). These results suggest that 12 WO Hets do have whisker-based tactile sensory processing deficits, which may be preventing the 12 WO SurHets from effectively performing the pup gathering paradigm. In order to show that PNNs are contributing to this tactile deficit in the Het, future studies should include enzymatic degradation of PNNs in the S1BF before tactile and pup gathering behaviors.

Is the atypical expression of PNNs in the SS1 causing tactile-based sensory deficits in the adult Het?

Through a time course analysis, we identified that the difference in SS1 PNN density between the Het and WT which is present at 12 WO and 10 WO, is not present at 6 WO. This indicates that there is a developmental shift occurring between these timepoints that is causing PNN expression to be dysregulated in the Het SS1. Within the RTT field, research is not often done using the female Het mouse model; when it

is, it is done once the mouse is considered symptomatic (around 16 weeks old), so there are not many developmental studies done on the pre-symptomatic Het (reviewed in Ribeiro and MacDonald, 2020).

Studies using *Mecp2-null* males found reduced dendritic complexity and smaller barrel representation in the S1BF (Lee et al., 2017; Moroto et al., 2013), similar morphological changes were seen in MECP2-negative cells of the motor cortex of the Het (Belichenko et al., 2009; Rietveld et al., 2015), though it has not been reported in the SS1. One study reported changes in the size of the Het S1BF throughout development, though the findings are trends (not significant data) they show that the S1BF area decreases in size at 7 WO when compared to WT, before normalizing back to WT at 14 WO (Smith et al., 2019). Due to the mosaic expression of MECP2, there may be neurons within the S1BF with these morphological differences but since all are not affected, the functional defects may take longer to appear when compared to the *Mecp2-null* males.

We found that 6 WO TacHets, those that had no atypical PNN density increase in the SS1 in the Naïve state, were able to perform the simple tactile tasks similarly to age-matched WTs, preferentially utilizing their whiskers to interact with the textures. Together, these data support our earlier speculation that increased PNN density is restricting plasticity in the SS1 of the 12 WO NaïveHet, resulting in tactile perception issues and inefficient pup retrieval. To further support this statement, we could enzymatically remove PNNs, through chondroitinase ABC injection, from the SS1 of the 12 WO NaïveHet and test their behavioral performance in the simple tactile behaviors. The removal of PNNs from cortical regions, via chondroitinase ABC injection, has been shown to re-establish plasticity to the region, allowing for a remodeling of the inhibitory network and rescue of the behavioral phenotype (Banerjee et al., 2017; Krishnan et al., 2017; Pizzorusso et al., 2002; Thompson et al., 2018). If the 12 WO TacHet were able to perform the simple tactile tasks after PNN degradation, this would support our speculation that the increased PNN density in the SS1 is causing deficits in tactile perception.

Without tactile perception deficits, will the 6 WO Hets be able to perform the pup gathering behavior?

As the Naïve 6 WO Het and WT have no PNN density differences in the SS1 and show no deficits in the simple tactile perception tasks, we expected them to be able to perform similarly to WT in the pup gathering assay. We report a robust phenotype within the etiologically relevant pup gathering behavior, where the 6 WO SurHet take significantly longer to retrieve pups back to the nest and have significantly more unsuccessful retrieval attempts compared to the SurWT, similar to the adult SurHet. We also found that the 6 WO SurHets, as a whole, were inefficient at the pup gathering assay on the first day of the behavior when compared to the SurWT performance (Figure 3.6 C & F). As the surrogates are cohoused with a novel adult (the pregnant mother) prior to the start of the gathering assay, there could already be plasticity alterations occurring in the SS1 of the Het before P0. These 6 WO behavioral assays show that MECP2 deficiency in the SS1 results in poor behavioral performance in a complex multisensory social context (pup gathering) but not in the simple tactile assay. This is similar to what was seen in the adult Het auditory cortex as the adult Hets had no issue with responding to and processing random sounds but did have an issue when presented with a contextually relevant pup calls (Krishnan et al., 2017; Lau et al., 2020a).

Together, these data suggest that MECP2 deficiency affects experience-dependent plasticity during complex multisensory paradigms, like the pup gathering behavior, but not during simple behaviors, like simple tactile assays. The pup gathering paradigm is complex, as it involves the integration of auditory, olfactory, and tactile sensory cues from multiple different pups throughout the experiment. On the other hand, the simple tactile assays only require the integration of tactile input. There are other, more simple multisensory paradigms, like the three-chamber social test or gap test, that involved limited exposure to novel adults and pups, (Bobrov et al., 2014; Lenschow and Brecht, 2015; Orefice et al., 2016; Rao et al., 2014), that could help us elucidate the context-specific nature of MECP2 function in the SS1.

Does MECP2 deficiency influence the formation of PNNs in the SS1 in a context-dependent manner?

In the adult SurHet auditory cortex, it was shown that inefficient pup gathering behavior was caused by the increased expression of PNNs as a result of the behavioral experience (Krishnan et al., 2017). Since the 6 WO Hets also did not perform the pup gathering behavior efficiently but performed in the simple tactile tasks, we conducted a whole brain quantification of PNN expression from both behavioral contexts. Our overall data showed that there is a significant dysregulation of PNN expression within the 6 WO SurHet SS1, but not in the 6 WO TacHet, SS1 after behavior.

Within the 6 WO TacHet brains, we found little to no change in PNN density when compared to the TacWT. There was a slight significant increase within the whole SS1, which was also present in the S1BF, S1FL, and S1DZ subregions (Table 3.4). However, when the densities were split between the left and right hemisphere, there was no significant alterations in PNN density (Table 3.4). Similar results were found when the PNN density of each region was split into top and bottom layers (summarized in Figure 3.12, C & D). As the significant increases in PNN density were so subtle, we decided to analyze each individual cohort (n = 4 per genotype) and found that one cohort was driving the significance in the TacHet (Figures 3.10 & 3.11). These results suggest that MECP2 deficiency does not affect PNN formation in the SS1 during the simple tactile experience.

In contrast to the 6 WO Tac context, the 6 WO Het underwent significant changes in PNN density after the pup gathering behavior within the SS1, in a subregion-, hemisphere- and layer-specific manner. Within the SS1, there is a significant PNN density increase within the SurHet when compared to the SurWT and the NaïveHet, which is consistent in the left and right hemisphere as well as the top and bottom layer PNN density measurements (Figure 3.7). Our whole SS1 data suggests that MECP2 deficiency is causing dysregulation of PNN formation in the SS1 during the complex multisensory paradigm.

Furthermore, we found that the SurHet had significantly higher PNN density than the SurWT and NaïveHet within the whole S1BF, as well as each hemisphere, which was also seen in both the top and bottom layer measurements (Figure 3.12 A &

B). These changes suggest that plasticity is being restricted in the S1BF, contributing to the inefficient pup retrieval which is supported by previous literature that states tactile input through the perioral area is important for this component of learned maternal paradigm (Morgan et al., 1992). Additionally, the increase is seen in the top and bottom layers, suggesting that tactile sensory input (layer IV), communication between other sensory regions (layers II & III) and information output (layers V & VI) is being dysregulated.

A similar result was seen the S1DZ, except the significant increase in SurHet PNN density compared to NaïveHet and SurWT is isolated to the left hemisphere. This hemispheric difference in PNN density between the SurHet and SurWT is only present in the top layers (Figure 3.12, A). Additionally, in the top layers, the SurHet has a significantly higher PNN density in the left hemisphere when compared to the right hemisphere. The top layers of the cortex (defined here as I-IV) are sensory input layers as well as cortico-cortical communication layers. Not much is known about the function of the S1DZ, but current studies suggest that it is an integration region for information from the SS1 as well as information from other sensory cortices (motor) that projects sensory information to sub-cortical regions (Chapin and Lin, 1984; Lee and Kim, 2012). The left hemisphere-specific increases in PNN density within SurHet, could suggest a restricted plasticity within this specific circuit.

The pup gathering behavior is a complex, multisensory paradigm, therefore we cannot state that the PNN density increase in the SurHet SS1 is the sole cause of the inefficient pup gathering phenotype in the 6 WO Het. We know that audition is involved in this behavior and that, in the adult Het, plasticity in the auditory cortex was also dysregulated (Krishnan et al., 2017). As we did not look at the auditory cortex in the 6 WO animals, we do not know if this was a contributing factor. In the future, investigating PNN expression within the auditory cortex of the 6 WO SurHet could lead to interesting long range connectivity studies between the two cortical regions as they pertain to social interactions (Rao et al., 2014) and pup gathering behavior.

With how we calculate PNN density, we acquire area data about the SS1 and its subregions. In the 6 WO SurHets, we found that the SS1 area of the SurHet

decreased significantly in comparison to both the SurWT and NaïveHet, but no change was seen between NaïveWT and NaïveHet. We found no reduction in the 6 WO TacHet SS1 area when compared to the 6 WO TacWT. The SS1 is a highly plastic region of the brain and has been shown to change in size depending on the amount of activity it is receiving (Kossut, 1998; Maier et al., 2003; Petersen, 2007; Posluszny et al., 2015; Rosselet et al., 2006; Xerri et al., 1994, 2005). Subregions that have significantly reduced areas in the SurHet, also have significantly higher PNN densities. Since these alterations are not seen in the SurWT, who performs the pup gathering behavior efficiently, this could suggest that inhibitory signaling is increased in these subregions. Follow up analysis could analyze the expression of inhibitory markers, like GAD67, PV or GABA which tend to be upregulated in the MECP2 mutant animals after a heightened sensory experience (Krishnan et al., 2015, 2017; Morello et al., 2018).

Our findings present further evidence that MECP2 is involved in the regulation of experience-dependent plasticity mechanisms, as measured by PNN expression, within the cortex in a context-specific manner. We present evidence that atypical increase of PNNs in the SS1 of the adult NaïveHet caused deficits in tactile perception, and that a similar increase in PNN density within the SS1 of 6 WO SurHet resulted in inefficient pup gathering behavior. Dysregulated plasticity mechanisms due to MECP2 deficiency led to context-dependent tactile-related sensory deficits within the 'pre-symptomatic' Het. This is consistent with the emerging literature on Rett Syndrome pathogenesis in patients, who begin to develop subtle sensory processing deficits before typical Rett Syndromic characteristics (Buchanan et al., 2019; Djukic and Valicenti McDermott, 2012; Nomura, 2005).

References

- Alsina-Llanes, M., Victoria De Brun, and Olazábal, D.E. (2015). Development and expression of maternal behavior in naïve female C57BL/6 mice: Ontogeny of Maternal Behavior in Mice. *Developmental Psychobiology* 57, 189–200.
- Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* 23, 185–188.
- Antunes, M., and Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process* 13, 93–110.
- Arkley, K., Tiktak, G.P., Breakell, V., Prescott, T.J., and Grant, R.A. (2017). Whisker touch guides canopy exploration in a nocturnal, arboreal rodent, the Hazel dormouse (*Muscardinus avellanarius*). *Journal of Comparative Physiology A* 203, 133–142.
- Aronoff, R., Matyas, F., Mateo, C., Ciron, C., Schneider, B., and Petersen, C.C.H. (2010). Long-range connectivity of mouse primary somatosensory barrel cortex: Long-range connectivity of barrel cortex. *European Journal of Neuroscience* 31, 2221–2233.
- Banerjee, S.B., Gutzeit, V.A., Baman, J., Aoued, H.S., Doshi, N.K., Liu, R.C., and Ressler, K.J. (2017). Perineuronal Nets in the Adult Sensory Cortex Are Necessary for Fear Learning. *Neuron* 95, 169-179.e3.
- Beach, F.A., and Jaynes, J. (1956). Studies of Maternal Retrieving in Rats. III. Sensory Cues Involved in the Lactating Female's Response To Her Young. *Behaviour* 10, 104–124.
- Bedogni, F., Cobolli Gigli, C., Pozzi, D., Rossi, R.L., Scaramuzza, L., Rossetti, G., Pagani, M., Kilstrup-Nielsen, C., Matteoli, M., and Landsberger, N. (2016). Defects During *Mecp2* Null Embryonic Cortex Development Precede the Onset of Overt Neurological Symptoms. *Cereb. Cortex* 26, 2517–2529.
- Belichenko, N.P., Belichenko, P.V., and Mobley, W.C. (2009). Evidence for both neuronal cell autonomous and nonautonomous effects of methyl-CpG-binding protein 2 in the cerebral cortex of female mice with *Mecp2* mutation. *Neurobiology of Disease* 34, 71–77.
- Berretta, S., Pantazopoulos, H., Markota, M., Brown, C., and Batzianouli, E.T. (2015). Losing the sugar coating: Potential impact of perineuronal net abnormalities on interneurons in schizophrenia. *Schizophrenia Research* 167, 18–27.
- Beurdeley, M., Spatazza, J., Lee, H.H.C., Sugiyama, S., Bernard, C., Di Nardo, A.A., Hensch, T.K., and Prochiantz, A. (2012). Otx2 Binding to Perineuronal Nets Persistently Regulates Plasticity in the Mature Visual Cortex. *Journal of Neuroscience* 32, 9429–9437.
- Bobrov, E., Wolfe, J., Rao, R.P., and Brecht, M. (2014). The Representation of Social Facial Touch in Rat Barrel Cortex. *Current Biology* 24, 109–115.

Bolte, S., and Cordelières, F.P. (2006). A guided tour into subcellular colocalization analysis in light microscopy. *J Microsc* 224, 213–232.

Brecht, M. (2017). The Body Model Theory of Somatosensory Cortex. *Neuron* 94, 985–992.

Buchanan, C.B., Stallworth, J.L., Scott, A.E., Glaze, D.G., Lane, J.B., Skinner, S.A., Tierney, A.E., Percy, A.K., Neul, J.L., and Kaufmann, W.E. (2019). Behavioral profiles in Rett syndrome: Data from the natural history study. *Brain and Development* 41, 123–134.

Cattaud, V., Bezzina, C., Rey, C.C., Lejards, C., Dahan, L., and Verret, L. (2018). Early disruption of parvalbumin expression and perineuronal nets in the hippocampus of the Tg2576 mouse model of Alzheimer's disease can be rescued by enriched environment. *Neurobiol. Aging* 72, 147–158.

Chahrour, M., and Zoghbi, H.Y. (2007). The Story of Rett Syndrome: From Clinic to Neurobiology. *Neuron* 56, 422–437.

Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T.C., Qin, J., and Zoghbi, H.Y. (2008). MeCP2, a Key Contributor to Neurological Disease, Activates and Represses Transcription. *Science* 320, 1224–1229.

Chao, H.-T., Chen, H., Samaco, R.C., Xue, M., Chahrour, M., Yoo, J., Neul, J.L., Gong, S., Lu, H.-C., Heintz, N., et al. (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* 468, 263–269.

Chapin, J.K., and Lin, C.S. (1984). Mapping the body representation in the SI cortex of anesthetized and awake rats. *J. Comp. Neurol.* 229, 199–213.

Chen, J.L., Carta, S., Soldado-Magraner, J., Schneider, B.L., and Helmchen, F. (2013). Behaviour-dependent recruitment of long-range projection neurons in somatosensory cortex. *Nature* 499, 336–340.

Cohen, L., Rothschild, G., and Mizrahi, A. (2011a). Multisensory integration of natural odors and sounds in the auditory cortex. *Neuron* 72, 357–369.

Cohen, S., Gabel, H.W., Hemberg, M., Hutchinson, A.N., Sadacca, L.A., Ebert, D.H., Harmin, D.A., Greenberg, R.S., Verdine, V.K., Zhou, Z., et al. (2011b). Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. *Neuron* 72, 72–85.

Dani, V.S., Chang, Q., Maffei, A., Turrigiano, G.G., Jaenisch, R., and Nelson, S.B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 102, 12560–12565.

Datavyu Team (2014). Datavyu: A Video Coding Tool. (Datavary Project, New York University).

Dellu, F., Mayo, W., Cherkaoui, J., Le Moal, M., and Simon, H. (1992). A two-trial memory task with automated recording: study in young and aged rats. *Brain Research* 588, 132–139.

- Dix, S.L., and Aggleton, J.P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behavioural Brain Research* 99, 191–200.
- Djukic, A., and Valicenti McDermott, M. (2012). Social Preferences in Rett Syndrome. *Pediatric Neurology* 46, 240–242.
- Durand, S., Patrizi, A., Quast, K.B., Hachigian, L., Pavlyuk, R., Saxena, A., Carninci, P., Hensch, T.K., and Fagiolini, M. (2012). NMDA receptor regulation prevents regression of visual cortical function in the absence of Mecp2. *Neuron* 76, 1078–1090.
- Ebert, D.H., Gabel, H.W., Robinson, N.D., Kastan, N.R., Hu, L.S., Cohen, S., Navarro, A.J., Lyst, M.J., Ekiert, R., Bird, A.P., et al. (2013). Activity-dependent phosphorylation of MeCP2 threonine 308 regulates interaction with NCoR. *Nature* 499, 341–345.
- Ennaceur, A. (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behavioural Brain Research* 215, 244–254.
- Ennaceur, A., and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research* 31, 47–59.
- Ennaceur, A., Michalikova, S., Bradford, A., and Ahmed, S. (2005). Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. *Behavioural Brain Research* 159, 247–266.
- Fagiolini, M., and Hensch, T.K. (2000). Inhibitory threshold for critical-period activation in primary visual cortex. *Nature* 404, 183–186.
- Feldman, D.E., and Brecht, M. (2005). Map plasticity in somatosensory cortex. *Science* 310, 810–815.
- Feldmeyer, D., Brecht, M., Helmchen, F., Petersen, C.C.H., Poulet, J.F.A., Staiger, J.F., Luhmann, H.J., and Schwarz, C. (2013). Barrel cortex function. *Progress in Neurobiology* 103, 3–27.
- Feldmeyer, D., Qi, G., Emmenegger, V., and Staiger, J.F. (2018). Inhibitory Interneurons and their Circuit Motifs in the Many Layers of the Barrel Cortex. *Neuroscience* 368, 132–151.
- Filipkowski, R.K. (2000). Tactile Experience Induces c-fos Expression in Rat Barrel Cortex. *Learning & Memory* 7, 116–122.
- Fleming, S.M. (2004). Early and Progressive Sensorimotor Anomalies in Mice Overexpressing Wild-Type Human α -Synuclein. *Journal of Neuroscience* 24, 9434–9440.
- Gabel, H.W., Kinde, B., Stroud, H., Gilbert, C.S., Harmin, D.A., Kastan, N.R., Hemberg, M., Ebert, D.H., and Greenberg, M.E. (2015). Disruption of DNA-methylation-dependent long gene repression in Rett syndrome. *Nature* 522, 89–93.
- Gogolla, N., Caroni, P., Lüthi, A., and Herry, C. (2009). Perineuronal nets protect fear memories from erasure. *Science* 325, 1258–1261.

- Grant, R.A., Mitchinson, B., Fox, C.W., and Prescott, T.J. (2009). Active touch sensing in the rat: anticipatory and regulatory control of whisker movements during surface exploration. *J. Neurophysiol.* 101, 862–874.
- Guić-Robles, E., Valdivieso, C., and Guajardo, G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. *Behavioural Brain Research* 31, 285–289.
- Guic-Robles, E., Jenkins, W.M., and Bravo, H. (1992). Vibrissal roughness discrimination is barrelcortex-dependent. *Behavioural Brain Research* 48, 145–152.
- Hartig, W., Brauer, K., and Bruckner, G. (1992). Wisteria floribunda agglutinin-labelled nets surround parvalbumin-containing neurons. *Neuroreport* 3, 869–872.
- von Heimendahl, M., Itskov, P.M., Arabzadeh, E., and Diamond, M.E. (2007). Neuronal Activity in Rat Barrel Cortex Underlying Texture Discrimination. *PLoS Biology* 5, e305.
- Helmchen, F., Gilad, A., and Chen, J.L. (2018). Neocortical Dynamics During Whisker-Based Sensory Discrimination in Head-Restrained Mice. *Neuroscience* 368, 57–69.
- Hou, X., Yoshioka, N., Tsukano, H., Sakai, A., Miyata, S., Watanabe, Y., Yanagawa, Y., Sakimura, K., Takeuchi, K., Kitagawa, H., et al. (2017). Chondroitin Sulfate Is Required for Onset and Offset of Critical Period Plasticity in Visual Cortex. *Scientific Reports* 7.
- Keniston, L.P., Henderson, S.C., and Meredith, M.A. (2010). Neuroanatomical identification of crossmodal auditory inputs to interneurons in somatosensory cortex. *Experimental Brain Research* 202, 725–731.
- Kenyon, P., Cronin, P., and Keeble, S. (1981). Disruption of maternal retrieving by perioral anesthesia. *Physiology & Behavior* 27, 313–321.
- Kinnischtzke, A.K., Simons, D.J., and Fanselow, E.E. (2014). Motor Cortex Broadly Engages Excitatory and Inhibitory Neurons in Somatosensory Barrel Cortex. *Cerebral Cortex* 24, 2237–2248.
- Kishi, N., and Macklis, J.D. (2004). MECP2 is progressively expressed in post-migratory neurons and is involved in neuronal maturation rather than cell fate decisions. *Molecular and Cellular Neuroscience* 27, 306–321.
- Knott, G.W., Quairiaux, C., Genoud, C., and Welker, E. (2002). Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. *Neuron* 34, 265–273.
- Kochlamazashvili, G., Henneberger, C., Bukalo, O., Dvoretzkova, E., Senkov, O., Lievens, P.M.-J., Westenbroek, R., Engel, A.K., Catterall, W.A., Rusakov, D.A., et al. (2010). The Extracellular Matrix Molecule Hyaluronic Acid Regulates Hippocampal Synaptic Plasticity by Modulating Postsynaptic L-Type Ca²⁺ Channels. *Neuron* 67, 116–128.
- Kossut, M. (1998). Experience-dependent changes in function and anatomy of adult barrel cortex. *Exp Brain Res* 123, 110–116.

- Krishnan, K., Wang, B.-S., Lu, J., Wang, L., Maffei, A., Cang, J., and Huang, Z.J. (2015). MeCP2 regulates the timing of critical period plasticity that shapes functional connectivity in primary visual cortex. *Proc. Natl. Acad. Sci. U.S.A.* *112*, E4782-4791.
- Krishnan, K., Lau, B.Y.B., Ewall, G., Huang, Z.J., and Shea, S.D. (2017). MECP2 regulates cortical plasticity underlying a learned behaviour in adult female mice. *Nature Communications* *8*, 14077.
- Kuhlman, S.J., O'Connor, D.H., Fox, K., and Svoboda, K. (2014). Structural Plasticity within the Barrel Cortex during Initial Phases of Whisker-Dependent Learning. *Journal of Neuroscience* *34*, 6078–6083.
- Lau, B.Y.B., Krishnan, K., Huang, Z.J., and Shea, S.D. (2020a). Maternal Experience-Dependent Cortical Plasticity in Mice Is Circuit- and Stimulus-Specific and Requires MECP2. *The Journal of Neuroscience* *40*, 1514–1526.
- Lau, B.Y.B., Layo, D.E., Emery, B., Everett, M., Kumar, A., Stevenson, P., Reynolds, K.G., Cherosky, A., Bowyer, S.-A.H., Roth, S., et al. (2020b). Lateralized Expression of Cortical Perineuronal Nets during Maternal Experience is Dependent on MECP2. *ENeuro* *7*, ENEURO.0500-19.2020.
- Layo, D.E., Lau, B.Y.B., and Krishnan, K. (2020). Mapping and Counting High-Intensity Perineuronal Nets in the Somatosensory Cortex v2 (protocols.io.bcf8itrw).
- Lee, T., and Kim, U. (2012). Descending projections from the dysgranular zone of rat primary somatosensory cortex processing deep somatic input. *J. Comp. Neurol.* *520*, 1021–1046.
- Lee, L.-J., Tsytsarev, V., and Erzurumlu, R.S. (2017). Structural and functional differences in the barrel cortex of *Mecp2* null mice. *Journal of Comparative Neurology* *525*, 3951–3961.
- Lee, S., Kruglikov, I., Huang, Z.J., Fishell, G., and Rudy, B. (2013). A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nature Neuroscience* *16*, 1662–1670.
- Lenschow, C., and Brecht, M. (2015). Barrel Cortex Membrane Potential Dynamics in Social Touch. *Neuron* *85*, 718–725.
- Lipp, H.P., and Van der Loos, H. (1991). A computer-controlled Y-maze for the analysis of vibrissotactile discrimination learning in mice. *Behav Brain Res* *45*, 135–145.
- Lonstein, J.S., Lévy, F., and Fleming, A.S. (2015). Common and divergent psychobiological mechanisms underlying maternal behaviors in non-human and human mammals. *Hormones and Behavior* *73*, 156–185.
- Maier, D.L., Grieb, G.M., Stelzner, D.J., and McCasland, J.S. (2003). Large-scale plasticity in barrel cortex following repeated whisker trimming in young adult hamsters. *Exp Neurol* *184*, 737–745.
- Mathis, M.W., Mathis, A., and Uchida, N. (2017). Somatosensory Cortex Plays an Essential Role in Forelimb Motor Adaptation in Mice. *Neuron* *93*, 1493-1503.e6.

- Miyata, S., and Kitagawa, H. (2017). Formation and remodeling of the brain extracellular matrix in neural plasticity: Roles of chondroitin sulfate and hyaluronan. *Biochimica et Biophysica Acta* 1861, 2420–2434.
- Morello, N., Schina, R., Pilotto, F., Phillips, M., Melani, R., Plicato, O., Pizzorusso, T., Pozzo-Miller, L., and Giustetto, M. (2018). Loss of Mecp2 Causes Atypical Synaptic and Molecular Plasticity of Parvalbumin-Expressing Interneurons Reflecting Rett Syndrome–Like Sensorimotor Defects. *Eneuro* 5, ENEURO.0086-18.2018.
- Morgan, H.D., Fleming, A.S., and Stern, J.M. (1992). Somatosensory control of the onset and retention of maternal responsiveness in primiparous Sprague-Dawley rats. *Physiology & Behavior* 51, 549–555.
- Moroto, M., Nishimura, A., Morimoto, M., Isoda, K., Morita, T., Yoshida, M., Morioka, S., Tozawa, T., Hasegawa, T., Chiyonobu, T., et al. (2013). Altered somatosensory barrel cortex refinement in the developing brain of Mecp2-null mice. *Brain Research* 1537, 319–326.
- Murthy, S., Kane, G.A., Katchur, N.J., Lara Mejia, P.S., Obiofuma, G., Buschman, T.J., McEwen, B.S., and Gould, E. (2019). Perineuronal Nets, Inhibitory Interneurons, and Anxiety-Related Ventral Hippocampal Neuronal Oscillations Are Altered by Early Life Adversity. *Biol. Psychiatry* 85, 1011–1020.
- Neul, J.L., Lane, J.B., Lee, H.-S., Geerts, S., Barrish, J.O., Annese, F., Baggett, L., Barnes, K., Skinner, S.A., Motil, K.J., et al. (2014). Developmental delay in Rett syndrome: data from the natural history study. *J Neurodev Disord* 6, 20.
- Nomura, Y. (2005). Early behavior characteristics and sleep disturbance in Rett syndrome. *Brain and Development* 27, S35–S42.
- O'Connor, D.H., Peron, S.P., Huber, D., and Svoboda, K. (2010). Neural Activity in Barrel Cortex Underlying Vibrissa-Based Object Localization in Mice. *Neuron* 67, 1048–1061.
- Orefice, L.L., Zimmerman, A.L., Chirila, A.M., Sleboda, S.J., Head, J.P., and Ginty, D.D. (2016). Peripheral Mechanosensory Neuron Dysfunction Underlies Tactile and Behavioral Deficits in Mouse Models of ASDs. *Cell* 166, 299–313.
- Pacchiarini, N., Fox, K., and Honey, R.C. (2017). Perceptual learning with tactile stimuli in rodents: Shaping the somatosensory system. *Learning & Behavior* 45, 107–114.
- Park, J.M., Hong, Y.K., Rodgers, C.C., Dahan, J.B., Schmidt, E.R., and Bruno, R.M. (2020). Deep and superficial layers of the primary somatosensory cortex are critical for whisker-based texture discrimination in mice (Neuroscience).
- Patrick Wu, H.-P., and Dyck, R.H. (2018). Signaling by Synaptic Zinc is Required for Whisker-Mediated, Fine Texture Discrimination. *Neuroscience* 369, 242–247.
- Paxinos, G., and Franklin, K.B.J. (2013). Paxino's and Franklin's The Mouse Brain In Stereotaxic Coordinates, 4th Ed (Waltham, MA: Academic Press).
- Petersen, C.C.H. (2007). The Functional Organization of the Barrel Cortex. *Neuron* 56, 339–355.

- Pizzo, R., Gurgone, A., Castroflorio, E., Amendola, E., Gross, C., Sassoè-Pognetto, M., and Giustetto, M. (2016). Lack of Cdkl5 Disrupts the Organization of Excitatory and Inhibitory Synapses and Parvalbumin Interneurons in the Primary Visual Cortex. *Front Cell Neurosci* 10, 261.
- Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J.W., and Maffei, L. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. *Science* 298, 1248–1251.
- Posluszny, A., Liguz-Leczna, M., Turzynska, D., Zakrzewska, R., Bielecki, M., and Kossut, M. (2015). Learning-Dependent Plasticity of the Barrel Cortex Is Impaired by Restricting GABA-Ergic Transmission. *PLoS ONE* 10, e0144415.
- Prigg, T., Goldreich, D., Carvell, G., and Simons, D. (2002). Texture discrimination and unit recordings in the rat whisker/barrel system. *Physiology & Behavior* 77, 671–675.
- Rao, R.P., Mielke, F., Bobrov, E., and Brecht, M. (2014). Vocalization–whisking coordination and multisensory integration of social signals in rat auditory cortex. *ELife* 3.
- Reinhard, S.M., Rais, M., Afroz, S., Hanania, Y., Pendi, K., Espinoza, K., Rosenthal, R., Binder, D.K., Ethell, I.M., and Razak, K.A. (2019). Reduced perineuronal net expression in Fmr1 KO mice auditory cortex and amygdala is linked to impaired fear-associated memory. *Neurobiol Learn Mem* 164, 107042.
- Ribeiro, M.C., and MacDonald, J.L. (2020). Sex differences in Mecp2-mutant Rett syndrome model mice and the impact of cellular mosaicism in phenotype development. *Brain Research* 1729, 146644.
- Richter, F., Gerstenberger, J., Bauer, A., Liang, C.-C., and Richter, A. (2017). Sensorimotor tests unmask a phenotype in the DYT1 knock-in mouse model of dystonia. *Behavioural Brain Research* 317, 536–541.
- Rietveld, L., Stuss, D.P., McPhee, D., and Delaney, K.R. (2015). Genotype-specific effects of Mecp2 loss-of-function on morphology of Layer V pyramidal neurons in heterozygous female Rett syndrome model mice. *Frontiers in Cellular Neuroscience* 9.
- Rodgers, C.C., Nogueira, R., Pil, B.C., Greeman, E.A., Park, J.M., Hong, Y.K., Fusi, S., and Bruno, R.M. (2021). Sensorimotor strategies and neuronal representations for shape discrimination. *Neuron* S089662732100372X.
- Rosset, C., Zennou-Azogui, Y., and Xerri, C. (2006). Nursing-Induced Somatosensory Cortex Plasticity: Temporally Decoupled Changes in Neuronal Receptive Field Properties Are Accompanied by Modifications in Activity-Dependent Protein Expression. *Journal of Neuroscience* 26, 10667–10676.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9, 676–682.
- Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675.

- Sigal, Y.M., Bae, H., Bogart, L.J., Hensch, T.K., and Zhuang, X. (2019). Structural maturation of cortical perineuronal nets and their perforating synapses revealed by superresolution imaging. *Proc. Natl. Acad. Sci. U.S.A.* *116*, 7071–7076.
- Şık, A., van Nieuwehuyzen, P., Prickaerts, J., and Blokland, A. (2003). Performance of different mouse strains in an object recognition task. *Behavioural Brain Research* *147*, 49–54.
- Skene, P.J., Illingworth, R.S., Webb, S., Kerr, A.R.W., James, K.D., Turner, D.J., Andrews, R., and Bird, A.P. (2010). Neuronal MeCP2 Is Expressed at Near Histone-Octamer Levels and Globally Alters the Chromatin State. *Molecular Cell* *37*, 457–468.
- Smith, E.S., Smith, D.R., Eyring, C., Braileanu, M., Smith-Connor, K.S., Ei Tan, Y., Fowler, A.Y., Hoffman, G.E., Johnston, M.V., Kannan, S., et al. (2019). Altered trajectories of neurodevelopment and behavior in mouse models of Rett syndrome. *Neurobiology of Learning and Memory* *165*, 106962.
- Sreenivasan, V., Karmakar, K., Rijli, F.M., and Petersen, C.C.H. (2015). Parallel pathways from motor and somatosensory cortex for controlling whisker movements in mice. *European Journal of Neuroscience* *41*, 354–367.
- Stefanelli, G., Gandaglia, A., Costa, M., Cheema, M.S., Di Marino, D., Barbiero, I., Kilstrup-Nielsen, C., Ausió, J., and Landsberger, N. (2016). Brain phosphorylation of MeCP2 at serine 164 is developmentally regulated and globally alters its chromatin association. *Sci Rep* *6*, 28295.
- Stern, J.M. (1990). Multisensory regulation of maternal behavior and masculine sexual behavior: A revised view. *Neuroscience & Biobehavioral Reviews* *14*, 183–200.
- Stern, J.M. (1996). Somatosensation and Maternal Care in Norway Rats. In *Advances in the Study of Behavior*, (Elsevier), pp. 243–294.
- Thompson, E.H., Lensjø, K.K., Wigestrang, M.B., Malthe-Sørenssen, A., Hafting, T., and Fyhn, M. (2018). Removal of perineuronal nets disrupts recall of a remote fear memory. *Proceedings of the National Academy of Sciences* *115*, 607–612.
- Ueno, H., Takao, K., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Okamoto, M., Aoki, S., and Ishihara, T. (2018). Age-dependent and region-specific alteration of parvalbumin neurons and perineuronal nets in the mouse cerebral cortex. *Neurochemistry International* *112*, 59–70.
- Welker, E., Rao, S.B., Dörfl, J., Melzer, P., and van der Loos, H. (1992). Plasticity in the barrel cortex of the adult mouse: effects of chronic stimulation upon deoxyglucose uptake in the behaving animal. *J. Neurosci.* *12*, 153–170.
- Wiest, M.C., Thomson, E., Pantoja, J., and Nicolelis, M.A.L. (2010). Changes in S1 Neural Responses During Tactile Discrimination Learning. *Journal of Neurophysiology* *104*, 300–312.
- Wright, E.A. (1965). The growth of mouse vibrissae. *Proc R Soc Med* *58*, 804–806.

Wu, H.-P.P., Ioffe, J.C., Iverson, M.M., Boon, J.M., and Dyck, R.H. (2013). Novel, whisker-dependent texture discrimination task for mice. *Behav. Brain Res.* 237, 238–242.

Xerri, C., Stern, J., and Merzenich, M. (1994). Alterations of the cortical representation of the rat ventrum induced by nursing behavior. *The Journal of Neuroscience* 14, 1710–1721.

Xerri, C., Bourgeon, S., and Coq, J.-O. (2005). Perceptual context-dependent remodeling of the forepaw map in the SI cortex of rats trained on tactile discrimination. *Behavioural Brain Research* 162, 207–221.

Xinhua Bao, Shengling Jiang, Fuying Song, Hong Pan, Meirong Li, and Wu, X.-R. (2008). X Chromosome Inactivation in Rett Syndrome and Its Correlations With MeCP2 Mutations and Phenotype. *J Child Neurol* 23, 22–25.

Zhou, Z., Hong, E.J., Cohen, S., Zhao, W., Ho, H.H., Schmidt, L., Chen, W.G., Lin, Y., Savner, E., Griffith, E.C., et al. (2006). Brain-Specific Phosphorylation of MeCP2 Regulates Activity-Dependent Bdnf Transcription, Dendritic Growth, and Spine Maturation. *Neuron* 52, 255–269.

Appendix

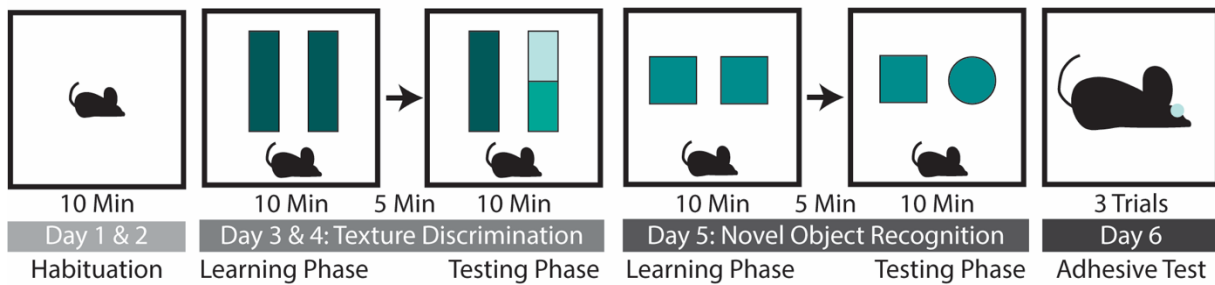
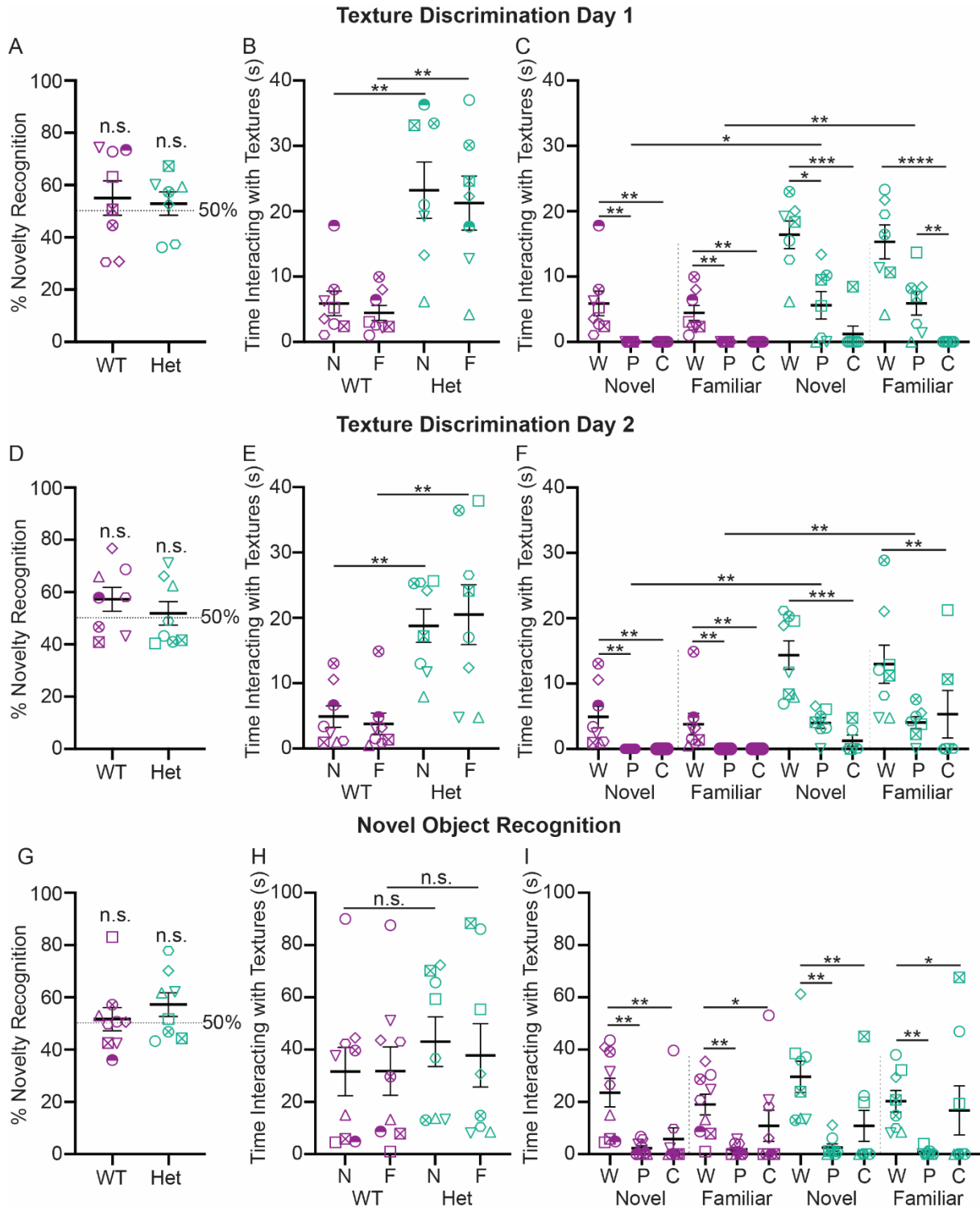


Figure 3.1. Simple Tactile Behavioral Assay Schema.

Habituation occurs on the first two days, to orient the mouse to the new behavior box. Texture discrimination is conducted on days three and four. During the learning phase, the mouse is familiarized to two identical textures. The mouse is then removed for 5 minutes and the textures are replaced with one identical to the textures in the learning phase and one new texture. During the testing phase, the time and way the mouse interacts with the textures is recorded. Each day tests a different new texture. On day five, the mouse goes through the novel object recognition task, which is similar to texture discrimination except the shapes change, not the textures. The final behavior is the adhesive test, where a sticker is placed on the nose of the mouse the efficacy of sticker removal is recorded, which is three trials.

Figure 3.2. 12 WO TacHets use compensatory mechanisms to interact with textures.

Behavioral performance in texture discrimination day 1 (A-C) and day 2 (D-F) as well as object recognition (G-I). Each shape indicates an individual mouse, within genotype, so the individual's behavior can be tracked throughout the assay. A, D, G. 12 WO TacHet (green) and TacWT (purple) do not show a novelty preference in any of the days. However, there is a lot of variability in the novelty preference percentages, with some animals being well above 50% (novelty) and others well below 50%, showing familiarity preference. **Texture Discrimination.** B, E. 12 WO TacHets spend significantly more time exploring both novel and familiar texture during texture discrimination day 1 and day 2. C, F. 12 WO TacHets use both their whiskers (w) and body (p+c) to explore the textures while the TacWTs do not. **Object Recognition.** H-I. 12 WO TacWT and TacHet perform similarly in this behavior based on time and the split between interacting with their whiskers, paws and climbing the textures. As these objects are 4x4x4 cm, the mice are easily able to climb and rest on top of them, while interacting with the other object with their whiskers. A, D, H. One sample t-test, 50% chance n.s.=not significant. B-C, E-F, H-I. *Kruskal-Wallis followed by uncorrected Dunn's test*, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Texture Discrimination Day 1, $n = 8$ WT and 7 Het ; Texture Discrimination Day 2, $n = 8$ WT and 8 Het ; Object Recognition, $n = 9$ WT and 8 Het.



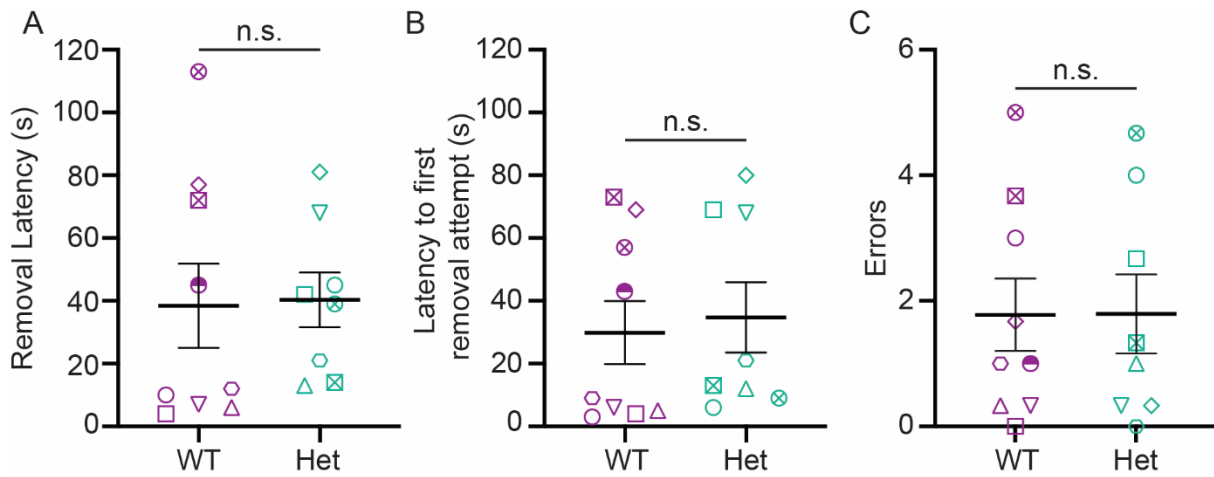


Figure 3.3. 12 WO TacHets do not show deficits in basic somatosensation through the adhesive test.

12 WO TacHets (purple) perform comparably to TacWTs (green) in adhesive test as measured by the time it takes for the mice to remove the adhesive (A), the latency to the first removal attempt (B), and the amount of unsuccessful removal attempts that occur after the first removal attempt (C). Each shape marks an individual mouse, within genotype, so the individual's behavior can be tracked throughout the assay; the same shapes mark the same mouse from Fig 3.2. Each dot represents the average performance of an individual mouse over 3 trials, $n = 9$ WT, 8 Het; *Mann-Whitney*, n.s. = no significance.

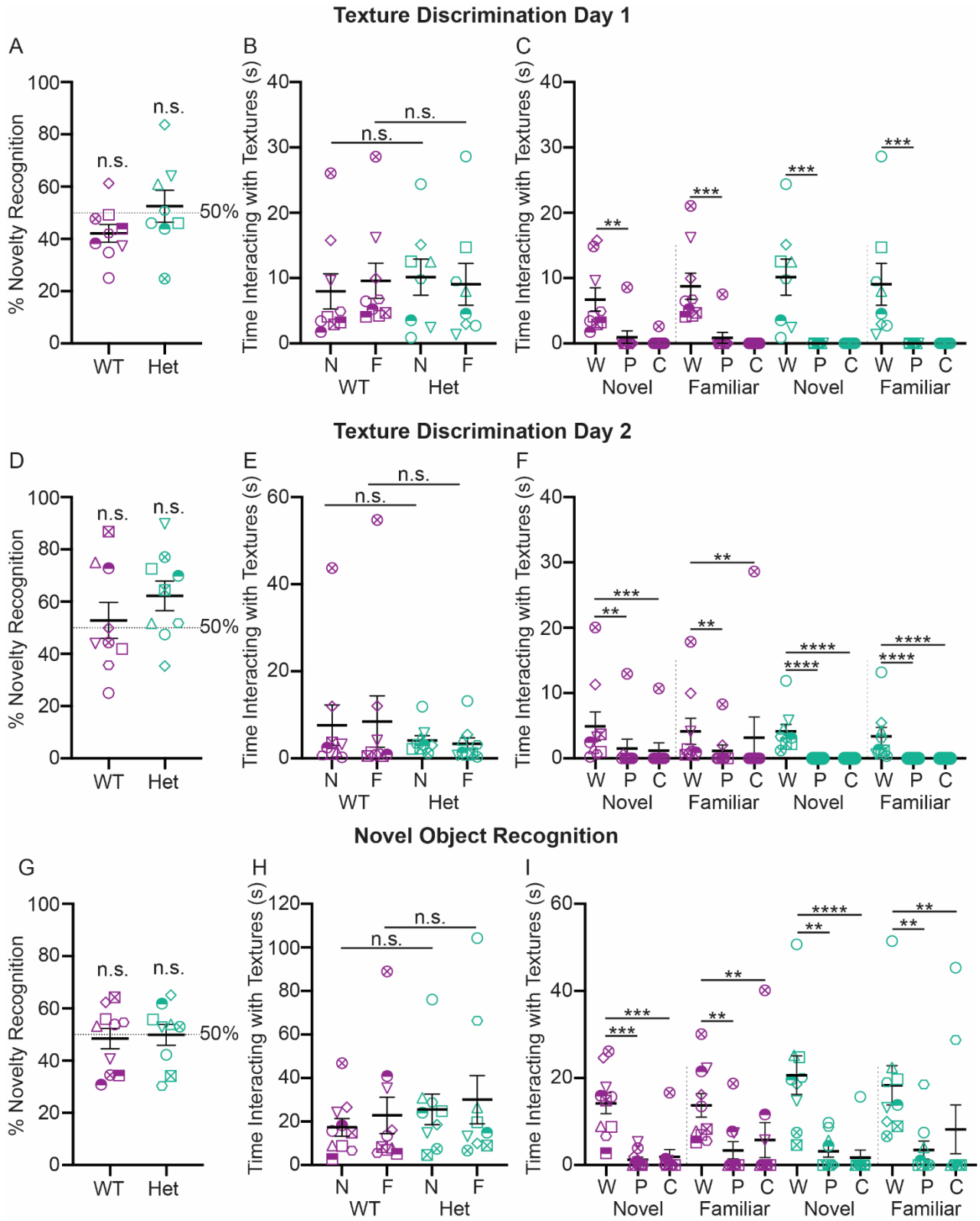
Table 3.1. A time course of the average high-intensity PNN density between the NaïveHet and NaïveWT within the SS1 and SS1 subregions.

Significant differences in PNN density between conditions within the same age are highlighted in green. Numbers correspond to average PNN density with standard error mean across multiple sections. When the subregions are analyzed separately, it becomes clear that PNN density was overall increased at 8 WO, in both Het and WT females, when compared to 12, 10 and 6 WO females. As these age groups are stained and imaged separately, this may be biological, or may be a technical artifact. Age groups here are imaged separately and the exposure settings for each age were different, as they were decided on based with age; making comparison between age groups difficult, as shown in Supplementary Figure 3.2. N = 24 – 46 sections per genotype per age spanning the SS1; 3 mice per age per genotype; Mann-Whitney comparison between genotypes within age group. SS1: N = 12 WO, WT = 45, Het = 45 :: 10 WO, WT = 46, Het = 46 :: 8 WO, WT = 39, Het = 40 :: 6 WO, WT = 40, Het = 39. S1BF: N = 12 WO, WT = 30, Het = 29 :: 10 WO, WT = 30, Het = 28 :: 8 WO, WT = 34, Het = 34 :: 6 WO, WT = 37, Het = 35. S1ULp: N = 12 WO, WT = 35, Het = 35 :: 10 WO, WT = 30, Het = 28 :: 8 WO, WT = 34, Het = 35 :: 6 WO, WT = 35, Het = 33. S1FL: N = 12 WO, WT = 25, Het = 25 :: 10 WO, WT = 25, Het = 24 :: 8 WO, WT = 25, Het = 25 :: 6 WO, WT = 24, Het = 24

	12 WO			10 WO			8 WO			6 WO	
	WT	Het		WT	Het		WT	Het		WT	Het
SS1	15.62 ± 1.5	25.58 ± 2.4		22.74 ± 2.1	30.21 ± 2.8		22.74 ± 2.1	30.21 ± 2.8		25.13 ± 3.0	26.11 ± 3.5
S1BF	23.91 ± 1.5	38.44 ± 3.2		30.64 ± 2.5	48.62 ± 3.6		69.69 ± 11.3	81.87 ± 7.3		31.90 ± 3.9	34.59 ± 5.0
S1ULp	14.72 ± 2.5	31.43 ± 4.3		23.19 ± 2.8	20.89 ± 4.1		50.49 ± 9.7	63.05 ± 9.7		22.14 ± 4.9	18.90 ± 3.8
S1FL	7.08 ± 1.2	13.22 ± 2.2		10.17 ± 2.1	15.72 ± 2.6		16.90 ± 4.1	31.16 ± 5.9		8.853 ± 2.2	8.722 ± 2.9

Figure 3.4. 6 WO TacHets use whiskers, not the body, to interact with textures in the whisker-based tactile tasks.

Behavioral performance in texture discrimination day 1 (A-C) and day 2 (D-F) as well as object recognition (G-I). Each shape indicates an individual mouse, within genotype, so the individual's behavior can be tracked throughout the assay. A, D, G. 6 WO TacHet (green) and TacWT (purple) do not show a novelty preference on texture discrimination day 1, day 2 or in the object recognition assay. Though TacWT shows a slight familiarization preference ($p=0.0505$) (A), while on day 2 there is a distinct split between novelty and familiarity preference in the TacWT and a trend towards novelty preferences in the TacHets (D). **Texture Discrimination.** 6 WO TacHets have similar exploration times compared with TacWT on texture discrimination day 1 (B) and day 2 (E). 6 WO TacHets solely use their whiskers to explore the textures (C, F), similar to TacWT. On both texture discrimination days, there is one TacWT outlier (circle with x) that uses its body to investigate textures (C, F). **Object Recognition.** G-I. 6 WO TacWT and TacHet perform similarly in this behavior based on time and the split between interacting with their whiskers, paws and climbing the textures. As these objects are 4x4x4 cm, the mice are easily able to climb and rest on top of them, while interacting with the other object with their whiskers. Across the days, the general outliers remained the TacWT, circle with x, and the TacHet, circle. A, D, G. One sample t-test, 50% chance n.s.=not significant. B-C, E-F, H-I. *Kruskal-Wallis followed by uncorrected Dunn's test*, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. Texture Discrimination Day 1, $n = 9$ WT and 7 Het ; Texture Discrimination Day 2, $n = 9$ WT and 9 Het ; Object Recognition, $n=10$ WT and 9 Het.



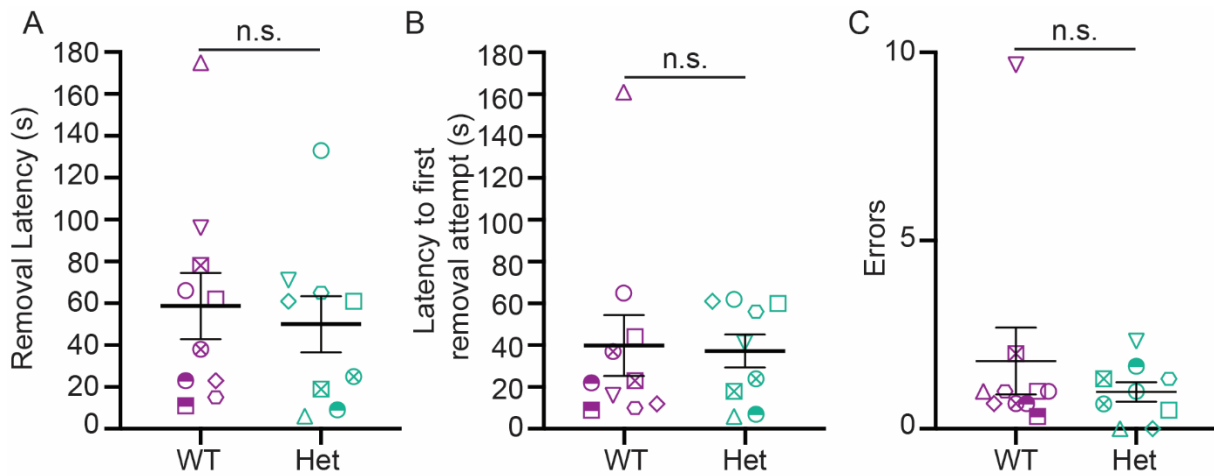
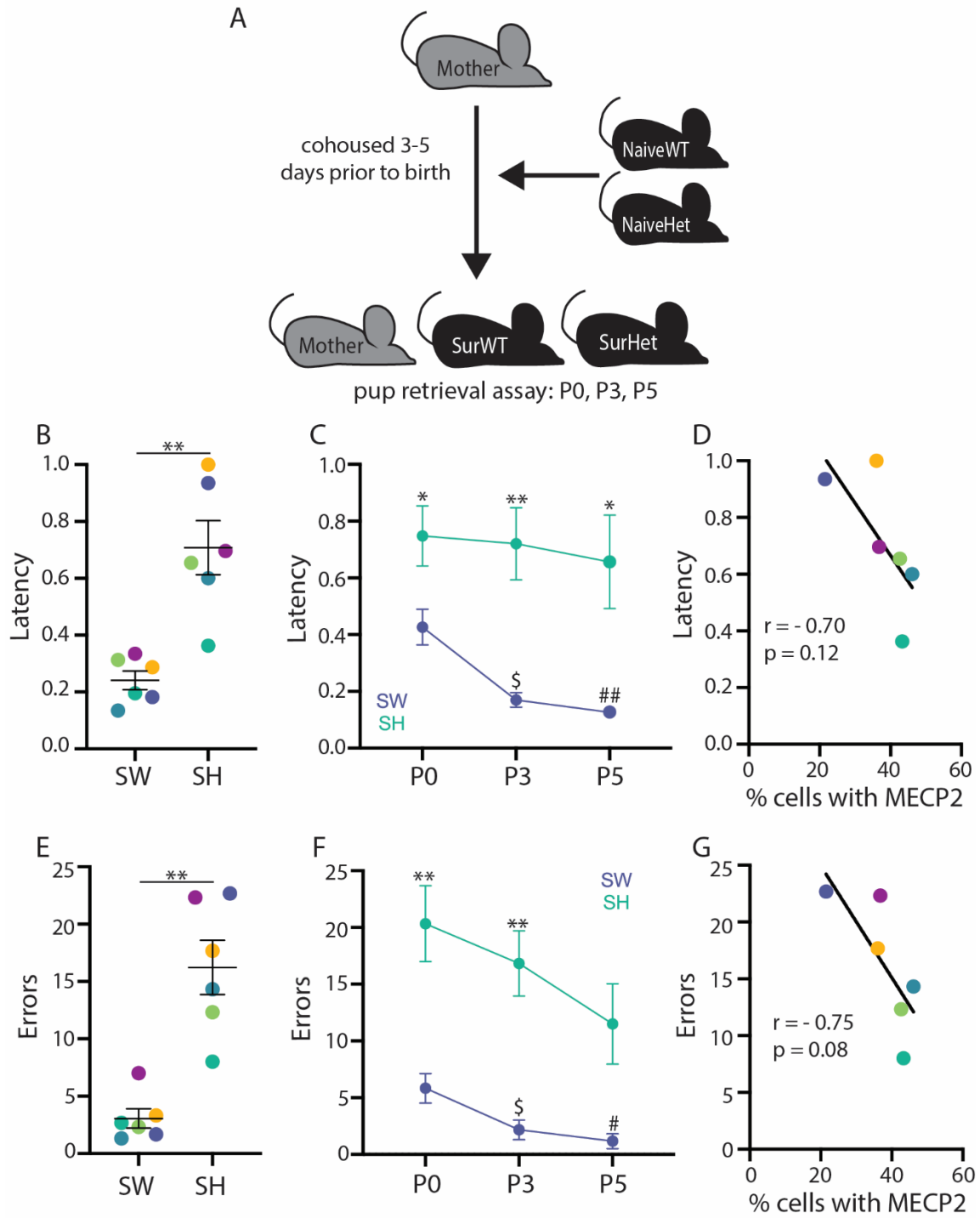


Figure 3.5. 6 WO TacHets do not show deficits in basic somatosensation through the adhesive test.

6 WO TacHets (green) perform comparably to TacWTs (purple) in adhesive test as measured by the time it takes for the mice to remove the adhesive (A), the latency to the first removal attempt (B), and the amount of unsuccessful removal attempts that occur after the first removal attempt (C). Each shape marks an individual mouse, within genotype, so the individual's behavior can be tracked throughout the assay; the same shapes mark the same mouse from Fig 3.4. Each dot represents the average performance of an individual mouse over 3 trials $n = 10$ WT, 9 Het; *Mann-Whitney*, n.s. = no significance.

Figure 3.6. 6 WO Surrogate Hets are inefficient at the pup gathering paradigm.

A. Schema of pup gathering paradigm. Two Naïve females, one wild-type (NW) and one *Mecp2-Het* (NH) are cohoused with a first-time pregnant CBA mother 3-5 days before birth; they are then termed 'surrogates'. The pup gathering behavior is performed on the day of birth (P0), P3 and P5 by the mother, surrogate WT (SW) and surrogate *Mecp2-Het* (SH). B, C, E, F. SH take longer to retrieve pups (latency, B, C) and have more errors in retrieval than SW (E, F). D, G. SH performance of latency (D) and errors (E) negatively correlate with percentage of the cell population expressing MECP2 (n= 6 Hets; Pearson's r). Each circle is an individual mouse, the color indicates cohort; variation seen within SH is shown in Supplementary Figure 3.3. B, E. Individual behavioral performance averaged over the three days. Each circle is an individual mouse, the color indicates SW and SH from the same cohort to highlight individual variability in the Het (n = 6); *Mann-Whitney test*, **p<0.01. C, F. Average behavioral performance of all animals within condition on each day. *Mann-Whitney test*, *p<0.05, **p<0.01; *between days within genotype*, *Kruskal-Wallis followed by Uncorrected Dunn's test*; P0 vs P3, \$p<0.05 : P0 vs P5 #p<0.05, ###p<0.01. B,C. **Latency.** SH take significantly longer on average to retrieve pups back to the nest (B). The SH also perform significantly worse than SW on each of the days (C) whereas the SW significantly improves overtime. E, F. **Errors.** The SH perform the pup gathering with significantly higher error when compared to SW on average (E) as well as on the first two days of behavior (F). On P5, SH show a slight, but not statistically significant improvement. SW improve significantly between P0 and P3 as well as P0 and P5.



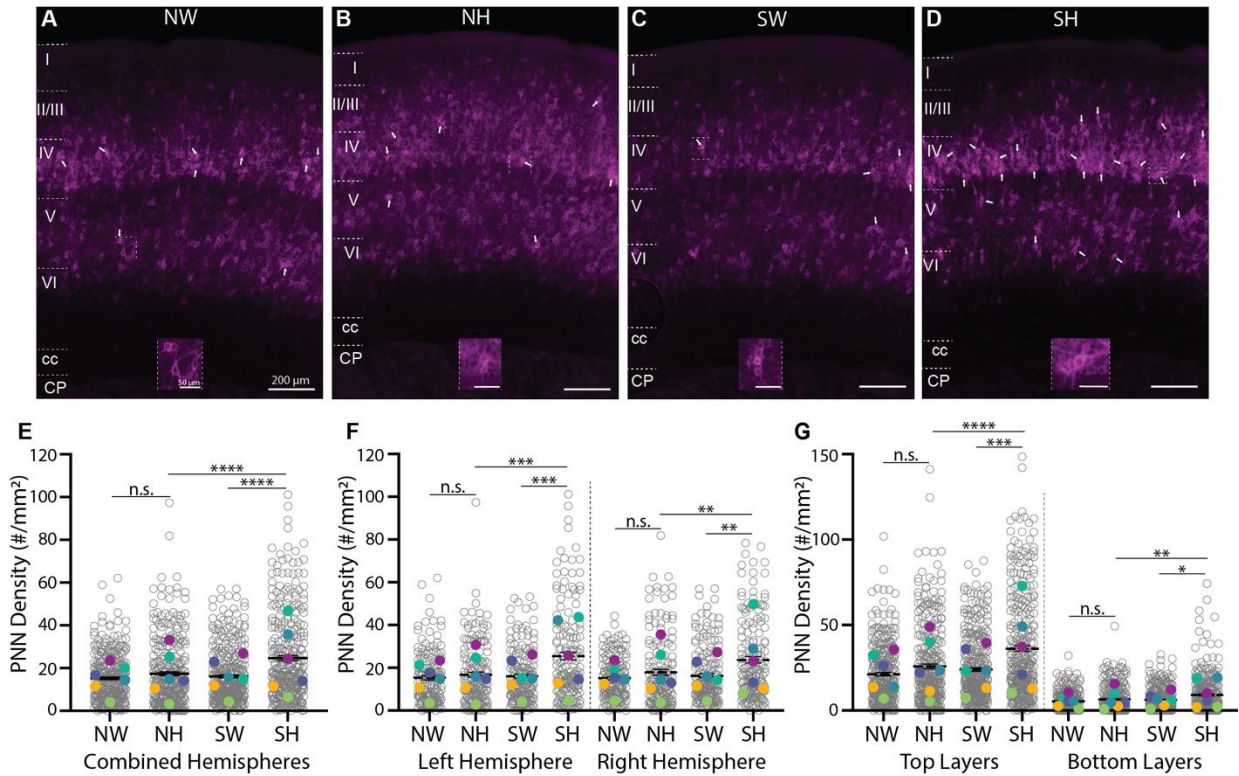


Figure 3.7. 6 WO Surrogate Het females show a significant increase in PNN density within the primary somatosensory cortex.

A-D. Representative images of PNN expression within the Primary Somatosensory Cortex of the NaïveWT (NW), NaïveHet (NH), SurWT (SW) and SurHet (SH). Insets in each show magnified PNN structures outlined in box. High-intensity PNNs are indicated by white arrows, cortical layers 1-6 are indicated on the right of each image; cc= corpus callosum, CP= caudate putamen. E-G. SH has significant increase in high-intensity PNN density when compared to both NH and SW (E), which is pervasive in each hemisphere (F) as well as the top and bottom layers (G). G. Top layers (layers I-IV) contain significantly more PNNs than bottom layers (layer V-VI); comparison not shown ($p < 0.0001$). Super plots show the average high-intensity PNN density, per cohort by the filled circles – different colors represent an individual cohort, which matches those indicated in Fig 3.6, overlaid on the gray open circles which represent individual sections. Some cohorts (yellow and lime green) have similar average PNN densities across conditions, while others (teal and purple) show variable expression between the 4 conditions. E-G. *Kruskal-Wallis followed by uncorrected Dunn's test*, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; n.s.=no significance. N= E+G. NW=307; NH=313; SW=339; SH=323 (G is per layer). F. Right: NW=153; NH=154; SW=173; SH=161; Left: NW=154; NH=159; SW=166; SH=162

Figure 3.8. SS1 PNN density is increased in a hemisphere- and subregion-specific manner in the SurHet

Whole (A-D) and hemisphere-specific (A'-D') high-intensity PNN density within the barrel field (BF), upper lip (ULp), dysgranular zone (DZ) and forelimb (FL) SS1 subregions. PNN densities are presented as violin plots (number of sections in each analysis below) overlayed with the average PNN density for each individual cohort (open-colored circles, colors match 3.6 and 3.7). Variable PNN density within cohort and between condition is more prevalent in the S1BF and S1ULp. A-A'. In the S1BF, SH has increased PNN density compared to SW and NH, which is contributed to by both hemispheres. B-B'. S1ULp has significant increase of PNN density after experience within the Het when compared to the WT, which is also seen in the right hemisphere of the S1ULp. C-C'. Like S1BF, SurHet has increased PNN density compared to both SW and NH in the S1DZ. This is seen exclusively in the left hemisphere of the S1DZ. SH has higher density of PNNs within the left hemisphere when compared to the right hemisphere. D-D'. SH has increased PNN density when compared to the SW in the S1FL. In the left hemisphere, there is increased PNN density in the Het after experience. In the right hemisphere, the WT has higher PNN density after experience. The NW has increased PNN density in the left hemisphere when compared to the right hemisphere. *Kruskal-Wallis followed by uncorrected Dunn's test*, **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. N = 6 mice per age per condition. S1BF N= Whole: NW=226; NH=230; SW=239; SH=243. Hemisphere: Left: NW=109; NH=118; SW=116; SH=121 :::: Right: NW=117; NH=112; SW=123; SH=122. S1ULp N= Whole: NW=263; NH=264; SW=291; SH=278. Hemisphere: Left: NW=136; NH=135; SW=139; SH=138 :::: Right: NW=127; NH=129; SW=152; SH=140. S1DZ N= Whole: NW=246; NH=248; SW=274; SH=263. Hemisphere: Left: NW=126; NH=128; SW=131; SH=132 :::: Right: NW=120; NH=120; SW=143; SH=131. S1FL Whole: NW=200; NH=203; SW=225; SH=210. Hemisphere: Left: NW=104; NH=106; SW=108; SH=106 :::: Right: NW=96; NH=97; SW=117; SH=104.

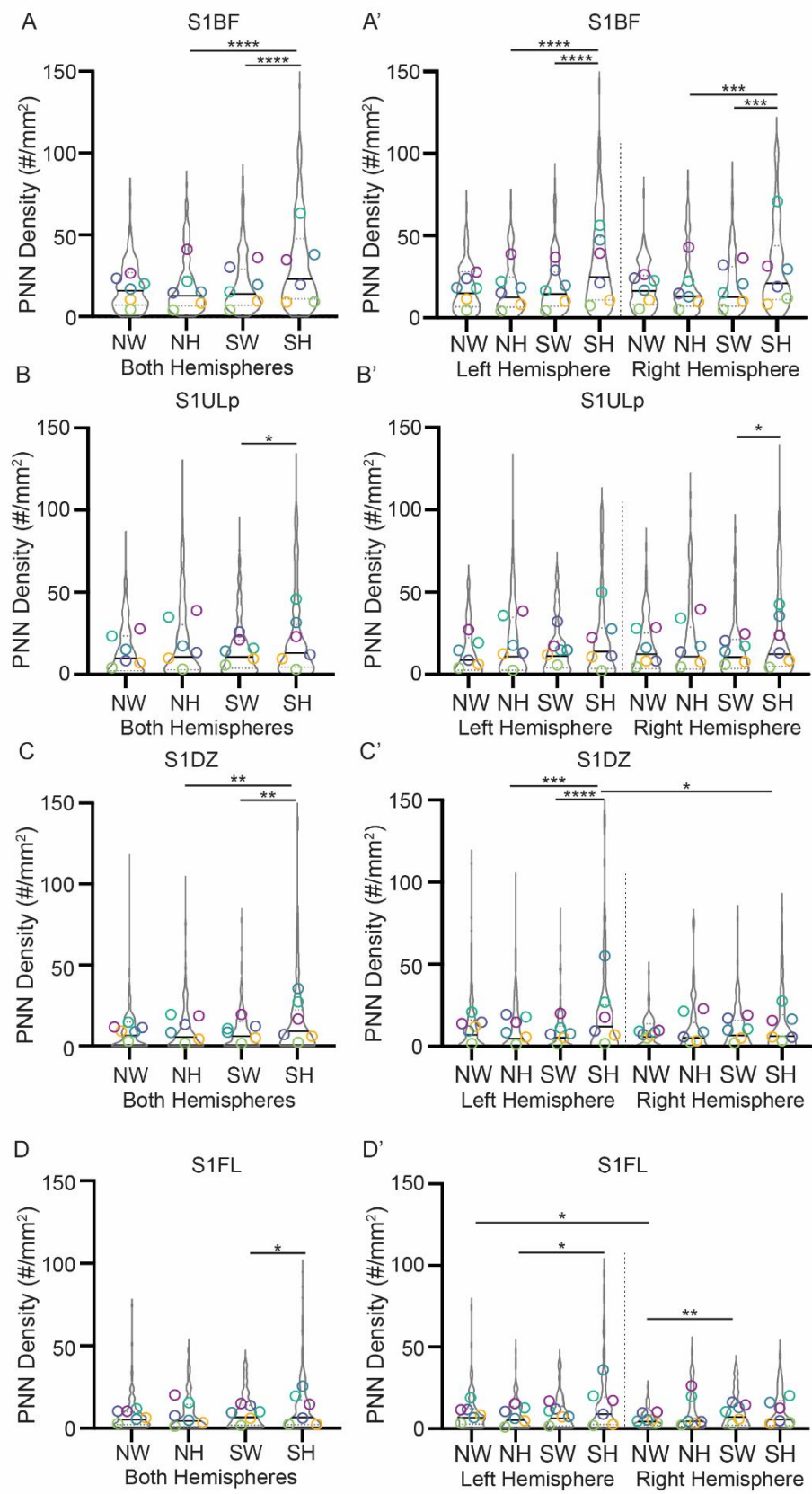


Table 3.2. PNN density differences within the top layers of the SS1 occur in a hemisphere and subregion-specific manner before and after pup gathering experience.

Significant differences in PNN density between genotypes, within the same condition, (ex. NW vs NH) are highlighted in blue. Significant differences between conditions within the same genotype (ex. NH vs SH) are **bolded**. Differences within animal between hemispheres (ex. NW right vs left hemisphere) are in *italics*. Numbers correspond to average PNN density with standard error mean across multiple sections. N = 96 - 152 images for hemisphere analysis, 291 - 200 images for combined hemisphere (for specifics see Fig 3.8) ; 6 mice per age per condition; *Kruskal-Wallis followed by uncorrected Dunn's test*.

Regions	Combined Hemisphere					Left Hemisphere					Right Hemisphere			
	NW	NH	SW	SH		NW	NH	SW	SH		NW	NH	SW	SH
S1BF	27.19 + 1.3	28.75 ± 1.7	29.87 ± 1.6	47.17 ± 2.5		22.83 + 1.8	24.21 ± 2.0	26.76 ± 2.1	42.74 ± 3.2		27.51 ± 1.8	29.91 ± 2.6	30.27 ± 2.3	46.71 ± 3.5
S1ULp	23.66 + 1.5	31.81 ± 2.2	23.62 ± 1.4	34.03 ± 2.3		21.11 + 1.9	31.58 ± 3.0	23.74 ± 2.0	32.87 ± 3.3		26.39 ± 2.3	32.06 ± 3.2	23.51 ± 2.0	35.17 ± 3.1
S1FL	13.41 + 1.1	14.54 ± 1.2	16.03 ± 1.1	20.66 ± 1.6		16.50 + 1.9	13.52 ± 1.6	15.25 ± 1.5	23.97 ± 2.7		10.06 ± 1.0	15.66 ± 1.8	16.75 ± 1.6	17.29 ± 1.7
S1DZ	17.01 + 1.5	19.59 ± 1.8	16.40 ± 1.3	28.30 ± 2.4		20.39 + 2.7	19.31 ± 2.4	15.25 ± 2.0	34.49 ± 4.0		11.95 ± 1.2	17.59 ± 2.3	15.71 ± 1.5	19.62 ± 2.2

Table 3.3. PNN density differences within the bottom layers of the SS1 occur in a hemisphere and subregion-specific manner before and after pup gathering experience.

Significant differences in PNN density between genotypes, within the same condition, (ex. NW vs NH) are highlighted in blue. Significant differences between conditions within the same genotype (ex. NH vs SH) are **bolded**. Differences within animal between hemispheres (ex. NW right vs left hemisphere) are in *italics*. Numbers correspond to average PNN density with standard error mean across multiple sections. N = 96 - 152 images for hemisphere analysis, 291 - 200 images for combined hemisphere (for specifics see Fig 3.8); 6 mice per age per condition; *Kruskal-Wallis followed by uncorrected Dunn's test*.

Region	Whole Brain				Left Hemisphere				Right Hemisphere			
	NW	NH	SW	SH	NW	NH	SW	SH	NW	NH	SW	SH
S1BF	6.67 ± 0.48	7.69 ± 0.59	7.71 ± 0.59	13.56 ± 0.9	6.64 ± 0.64	7.20 ± 0.74	7.65 ± 0.79	14.62 ± 1.4	6.41 ± 0.60	7.37 ± 0.83	8.34 ± 0.86	12.70 ± 1.2
S1ULp	5.70 ± 0.53	8.52 ± 0.79	6.50 ± 0.56	10.84 ± 1.0	5.12 ± 0.66	8.73 ± 1.11	6.66 ± 0.78	10.76 ± 1.4	6.31 ± 0.85	8.30 ± 1.14	6.34 ± 0.80	10.93 ± 1.4
S1FL	2.14 ± 0.28	3.29 ± 0.45	2.90 ± 0.36	4.62 ± 0.71	2.56 ± 0.39	3.57 ± 0.49	3.08 ± 0.47	6.34 ± 1.00	1.19 ± 0.23	4.63 ± 0.81	3.08 ± 0.46	3.97 ± 0.94
S1DZ	2.28 ± 0.43	3.09 ± 0.55	3.02 ± 0.54	5.6 ± 0.88	3.39 ± 0.67	4.11 ± 0.70	3.60 ± 0.56	7.52 ± 1.26	2.14 ± 0.49	3.69 ± 0.87	3.87 ± 0.73	5.14 ± 0.94

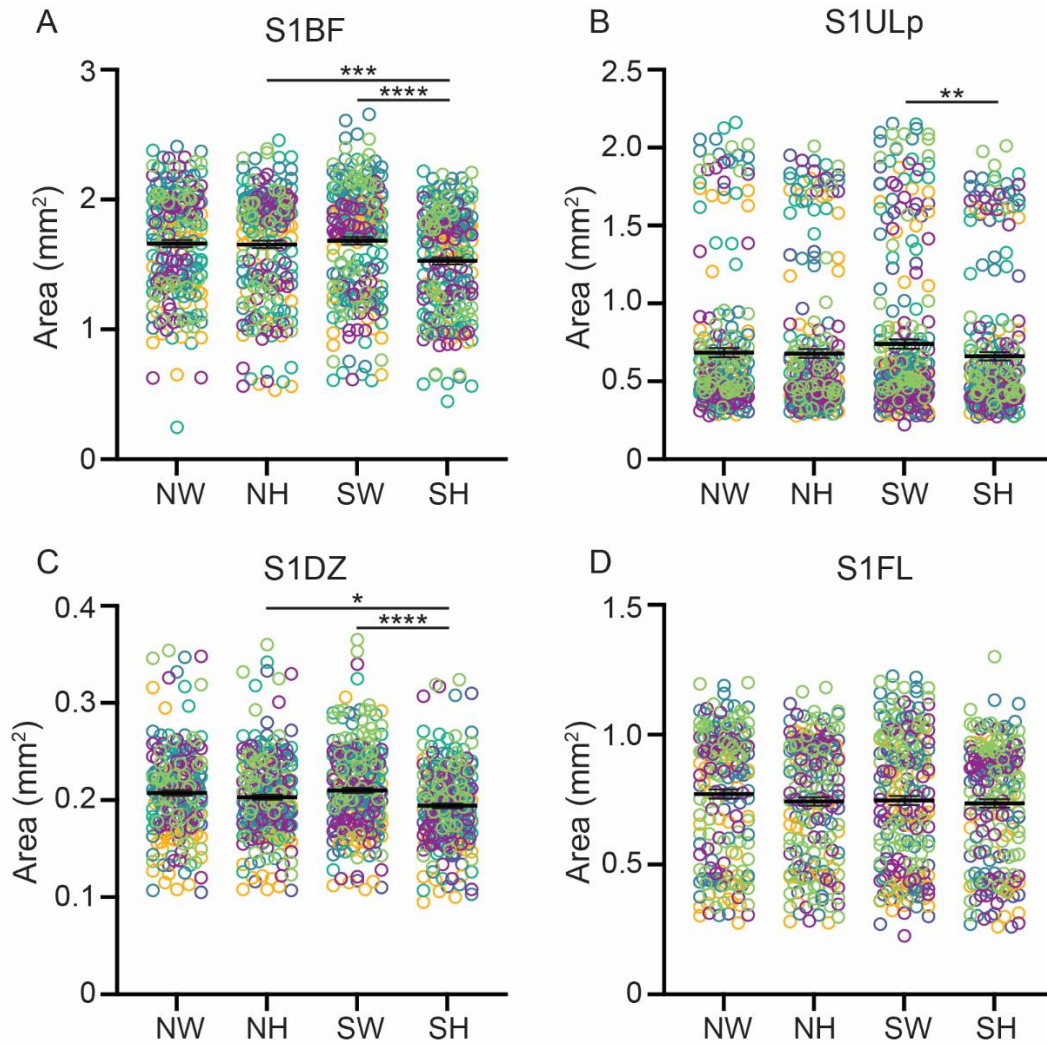


Figure 3.9. Reduction in specific subregion areas in SurHet SS1 after pup gathering behavior.

A, C. SH has reduced area compared to SW and NH in the S1BF and S1DZ. B. S1ULp has a significant decrease in area after experience in the Het when compared to the WT. D. Unlike the other regions, there are no differences in area in the S1FL. A-D. Each circle is a section, color coded by cohort (following the color schema from 3.6). No specific animal or cohort is driving the significant reduction in SH SS1 areas. *Kruskal-Wallis followed by uncorrected Dunn's test*, **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. N = 6 mice per age per condition. N= A. NW=226; NH=230; SW=239; SH=243. B. NW=263; NH=264; SW=261; SH=278. C. NW=245; NH=247; SW=273; SH=263, D. NW=199; NH=203; SW=225; SH=210

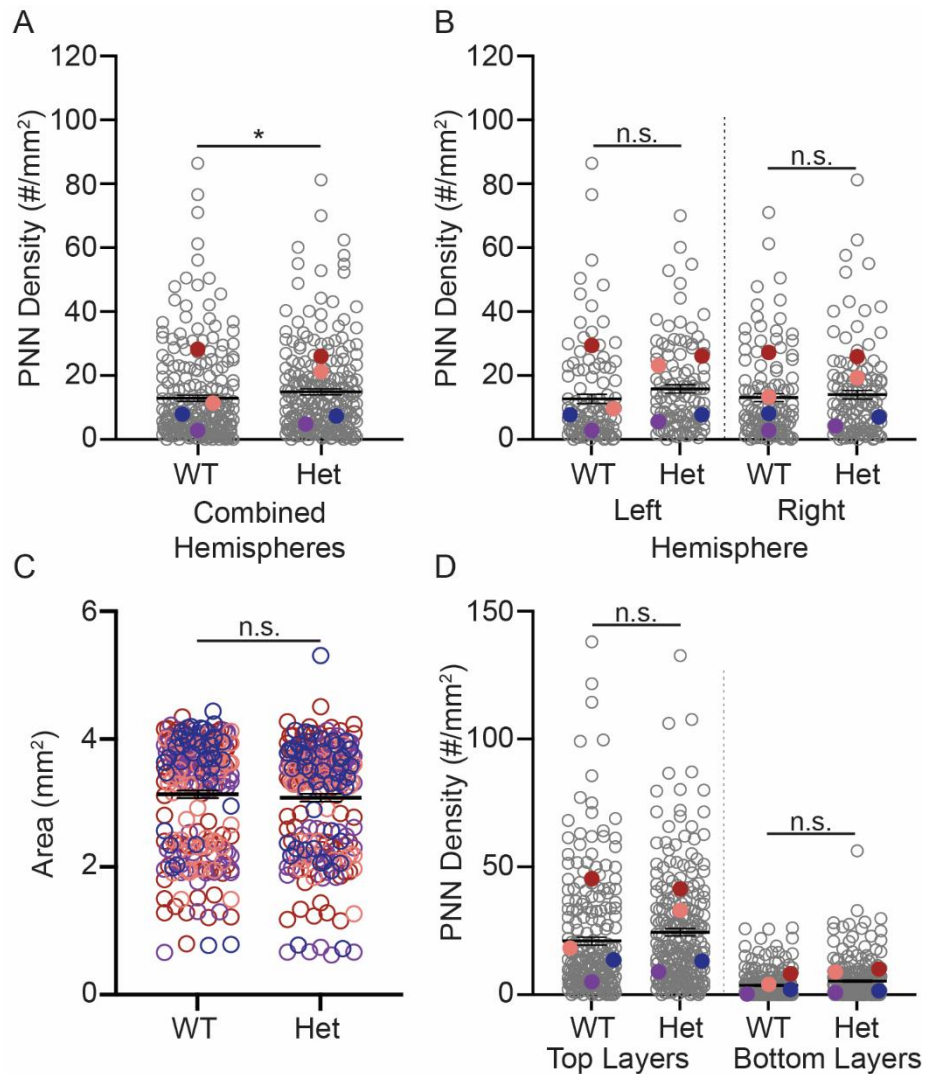


Figure 3.10. 6 WO TacHet show subtle alterations in PNN density within SS1.

A+B. 6 WO TacHet has a slightly higher PNN density in the combined SS1 when compared to TacWT (A), however this significant difference is not present when the hemispheres are split (B). The filled circles superimposed onto the graphs, represent the average PNN density per cohort ($n = 4$). This shows that the slight significant difference in PNN density seen in the SS1 is mainly contributed by one cohort (peach). C. Overall, there are no significant changes seen in the SS1 area between TacHet and TacWT. D. The significant change seen in the combined SS1 is not pervasive in either the top or bottom layers. Regardless of genotype, top layers have significantly higher density of PNNs than the bottom layers (not shown, $p < 0.0001$). A+C. *Mann-Whitney test*, $*p < 0.05$. B+D. *Kruskal-Wallis followed by Dunn's test*, n.s. = not significant. A,B,D. Each open circle is a section. C. Each open circle is an area measurement for a section, color coded by cohort, $n = 4$ mice per genotype. $N = 227$ WT, 228 Het. (B) $n =$ Left WT=111; Het=111; Right WT=116; Het=117.

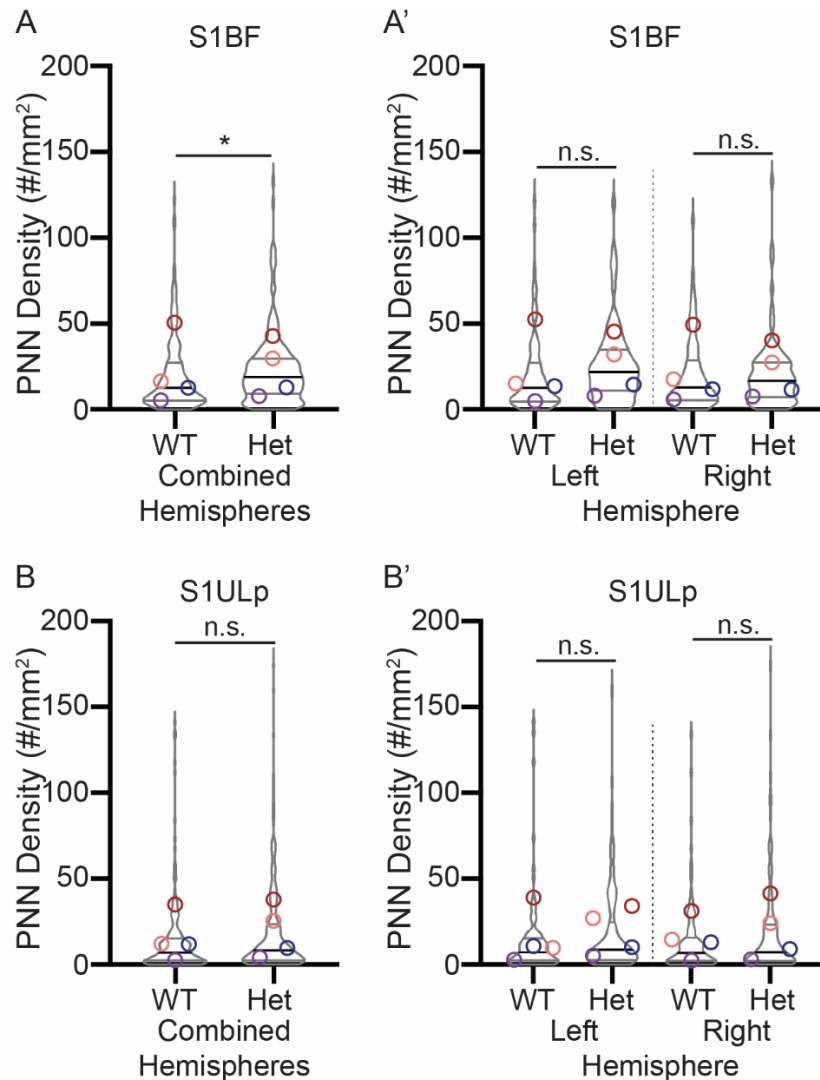


Figure 3.11. After the simple tactile assay, 6 WO TacHet have slight increased PNN density in specific subregions, but not in others.

Examples of subregions that do, S1BF (A) and do not, S1ULp (B), undergo changes in PNN density after tactile behavior, representative of other subregions described in Table 3.4. A-A'. 6 WO TacHet have slight increases in PNN density within the S1BF after the simple tactile assay, which is not present when the hemispheres are split; similar to S1FL and S1DZ (Table 3.4). B-B'. No significant differences in PNN density are seen in the S1ULp after simple tactile behavior; similar to S1J, S1HL, S1Tr and S1Sh (Table 3.4). PNN densities are presented as violin plots (number of sections in each analysis below) overlaid with the average PNN density for each individual cohort (open-colored circles). A+B. *Mann-Whitney*, $*p < 0.05$. A'+B'. *Kruskal-Wallis followed by Dunn's test*, n.s. = not significant. N = 4 animals per genotype. A-A'. Whole: W=168; H=164 A'. Hemisphere: Left: W=83; H=82::: Right: W=85; H=83. B-B'. Whole: W=192; H=208 A'. Hemisphere: Left: W=95; H=100::: Right: W=97; H=108

Table 3.4. PNN density alterations in the 6 WO Tac SS1 occur within specific subregions, but not within individual hemispheres.

Significant differences in PNN density between genotypes are highlighted in orange. Numbers correspond to average PNN density with standard error mean across multiple sections. S1BF, S1FL and S1DZ show subtle increases in PNN density within the TacHet compared to TacWT after behavior, though it is not significant when the hemispheres are split. S1ULp, S1J, S1HL, S1Tr and S1Sh do not show any difference in PNN density between TacWT and TacHet after behavior. N = 11 - 90 images for hemisphere analysis, 25 - 180 images for combined hemisphere; 6 mice per age per condition; *Mann-Whitney* for combined hemisphere analysis, *Kruskal-Wallis followed by Dunn's test* for split hemispheres. N= S1BF. Whole: W=168; H=164:: Hemisphere: Left: W=83; H=82 :::: Right: W=85; H=83. S1ULp. Whole: W=192; H=208:: Hemisphere: Left: W=95; H=100 :::: Right: W=97; H=108. S1FL. Whole: W=150; H=151:: Hemisphere: Left: W=75; H=75 :::: Right: W=75; H=76. S1DZ. Whole: W=180; H=177:: Hemisphere: Left: W=90; H=87::: Right: W=90; H=90. S1J. Whole: W=48; H=48:: Hemisphere: Left: W=25; H=22::: Right: W=23; H=26. S1HL. Whole: W=135; H=137:: Hemisphere: Left: W=68; H=68 :::: Right: W=67; H=69. S1Tr. Whole: W=25; H=29:: Hemisphere: Left: W=11; H=13::: Right: W=14; H=16. S1Sh. Whole: W=31; H=32:: Hemisphere: Left: W=15; H=16 :::: Right: W=16 ; H=16

Regions	Combined Hemisphere			Left Hemisphere			Right Hemisphere	
	TacWT	TacHet		TacWT	TacHet		TacWT	TacHet
S1BF	20.58 ± 1.7	23.67 ± 1.74		20.68 ± 2.64	26.12 ± 2.52		20.48 ± 2.29	19.52 ± 2.88
S1ULp	14.58 ± 1.8	19.44 ± 1.96		14.70 ± 2.54	19.36 ± 2.65		14.45 ± 2.27	32.87 ± 3.33
S1FL	5.93 ± 0.91	7.92 ± 1.03		5.37 ± 1.46	8.46 ± 1.61		6.50 ± 1.09	7.38 ± 1.29
S1DZ	8.46 ± 1.16	11.48 ± 1.16		7.59 ± 1.58	13.47 ± 2.27		9.33 ± 1.69	9.33 ± 9.56
S1J	14.78 ± 3.20	18.28 ± 3.22		10.63 ± 3.04	16.82 ± 4.29		19.28 ± 5.72	19.52 ± 4.77
S1HL	0.762 ± 0.16	0.96 ± 0.17		0.72 ± 0.24	0.73 ± 0.18		0.81 ± 0.21	1.18 ± 0.29
S1Tr	2.44 ± 0.88	3.91 ± 1.67		1.89 ± 1.00	2.40 ± 0.65		2.87 ± 1.38	5.14 ± 2.98
S1Sh	6.00 ± 1.32	4.04 ± 0.93		4.48 ± 1.22	3.94 ± 1.40		7.42 ± 2.29	4.12 ± 1.27

Table 3.5. PNN density alterations within the top layers of SS1 sub-regions in the 6 WO TacHet.

Significant differences in PNN density between genotypes are highlighted in orange. Numbers correspond to average PNN density with standard error mean across multiple sections. S1BF and S1FL show subtle increases in PNN density within the TacHet compared to TacWT after behavior, though it is not significant when the hemispheres are split. S1DZ, S1ULp, S1J, S1HL, S1Tr and S1Sh do not show any difference in PNN density between TacWT and TacHet after behavior. N = 11 - 90 images for hemisphere analysis, 25 - 180 images for combined hemisphere (for specifics see Table 3.4); 6 mice per age per condition; *Mann-Whitney* for combined hemisphere analysis, *Kruskal-Wallis followed by Dunn's test* for split hemispheres.

Regions	Combined Hemisphere			Left Hemisphere			Right Hemisphere	
	TacWT	TacHet		TacWT	TacHet		TacWT	TacHet
S1BF	32.75 ± 2.65	38.01 ± 2.63		32.62 ± 3.95	39.89 ± 3.73		32.87 ± 3.71	36.26 ± 3.71
S1ULp	24.26 ± 2.84	30.12 ± 2.94		24.60 ± 4.32	30.36 ± 4.08		23.92 ± 3.08	29.90 ± 4.23
S1FL	10.50 ± 1.56	13.60 ± 1.54		9.27 ± 2.44	13.68 ± 2.30		11.73 ± 1.95	13.51 ± 2.07
S1DZ	14.34 ± 1.94	18.94 ± 2.55		12.33 ± 2.52	22.32 ± 3.92		2.94 ± 2.94	15.90 ± 3.21
S1J	23.15 ± 5.10	28.29 ± 4.99		16.49 ± 4.83	26.78 ± 7.26		30.39 ± 9.15	29.56 ± 7.10
S1HL	1.45 ± 0.31	1.71 ± 0.31		1.32 ± 0.45	1.26 ± 0.30		1.578 ± 0.43	2.15 ± 0.54
S1Tr	3.75 ± 1.34	5.86 ± 2.12		3.41 ± 1.45	8.64 ± 4.19		4.06 ± 2.25	3.25 ± 1.06
S1Sh	11.94 ± 2.44	7.95 ± 1.78		7.81 ± 2.05	6.75 ± 2.17		12.82 ± 3.87	7.34 ± 2.17

Table 3.6. PNN density alterations within the bottom layers of SS1 sub-regions in the 6 WO TacHet.

Significant differences in PNN density between genotypes are highlighted in orange. Numbers correspond to average PNN density with standard error mean across multiple sections. S1BF, S1ULp and S1FL show subtle increases in PNN density within the TacHet compared to TacWT after behavior, though it is not significant when the hemispheres are split. All other subregions do not show any difference in PNN density between TacWT and TacHet after behavior. N = 11 - 90 images for hemisphere analysis, 25 - 180 images for combined hemisphere (for specifics see Table 3.4); 6 mice per age per condition; *Mann-Whitney* for combined hemisphere analysis, *Kruskal-Wallis* followed by *Dunn's test* for split hemispheres.

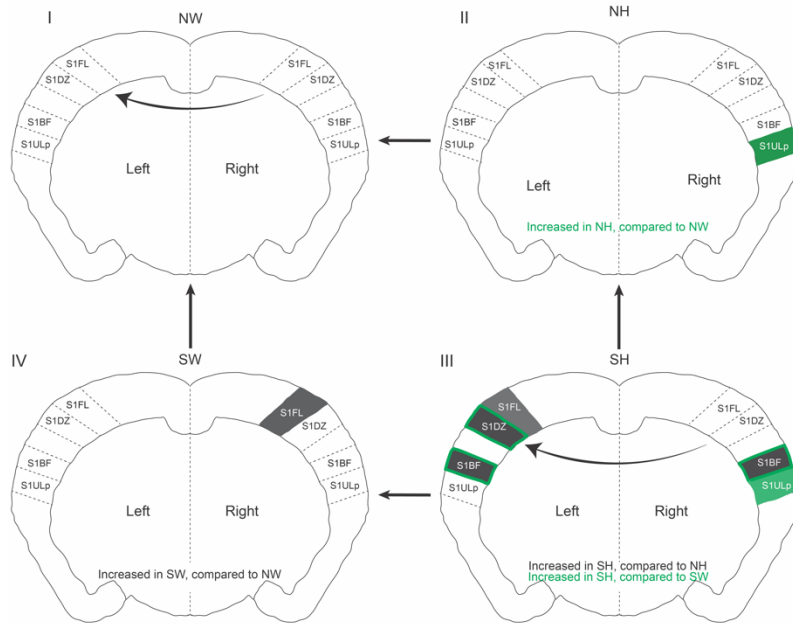
Regions	Combined Hemisphere			Left Hemisphere			Right Hemisphere	
	TacWT	TacHet		TacWT	TacHet		TacWT	TacHet
S1BF	5.70 $\pm$ 0.68	8.02 $\pm$ 0.78		6.16 $\pm$ 1.09	9.48 $\pm$ 1.29		5.24 $\pm$ 0.838	6.69 $\pm$ 0.92
S1ULp	4.31 $\pm$ 0.68	7.54 $\pm$ 1.02		3.87 $\pm$ 0.77	7.121 $\pm$ 1.36		4.74 $\pm$ 1.11	7.92 $\pm$ 1.51
S1FL	1.24 $\pm$ 0.26	2.96 $\pm$ 0.52		1.178 $\pm$ 0.36	3.26 $\pm$ 0.91		1.29 $\pm$ 0.376	2.67 $\pm$ 0.54
S1DZ	2.31 $\pm$ 0.48	3.03 $\pm$ 0.62		2.40 $\pm$ 0.75	3.67 $\pm$ 0.94		2.21 $\pm$ 0.60	2.67 $\pm$ 0.82
S1J	4.32 $\pm$ 0.99	6.15 $\pm$ 1.46		3.11 $\pm$ 0.97	3.85 $\pm$ 1.11		5.63 $\pm$ 1.76	8.10 $\pm$ 2.48
S1HL	0.06 $\pm$ 0.03	0.21 $\pm$ 0.09		0.04 $\pm$ 0.04	0.21 $\pm$ 0.14		0.08 $\pm$ 0.05	0.20 $\pm$ 0.10
S1Tr	0.32 $\pm$ 0.22	1.31 $\pm$ 0.98		0.67 $\pm$ 0.45	2.71 $\pm$ 2.01		0.30 $\pm$ 0.30	0 $\pm$ 0
S1Sh	0.60 $\pm$ 0.29	0.36 $\pm$ 0.36		0.23 $\pm$ 0.23	0.58 $\pm$ 0.58		0.80 $\pm$ 0.45	0 $\pm$ 0

Figure 3.12. Summary of changes in PNN density by layer at 6 WO within the pup gathering and simple tactile behaviors.

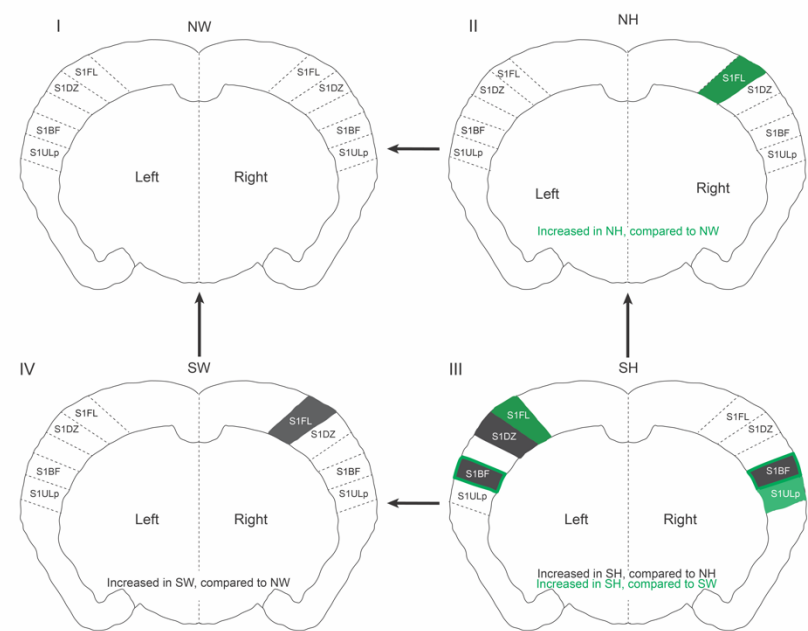
Alterations in PNN density during the pup gathering behavior (A-B) and after the simple tactile paradigm (C, D) in the top (A, C) and bottom (B, D) layers of the SS1. PNN density changes between conditions, marked by external arrows, are indicated by shading within the increased subregion (green: within condition; gray: within genotype). Arrows inside the brain indicate hemispheric subregion differences within brain (pointing at increased hemisphere). C, D. Within the simple tactile behavior, there are no hemispheric alterations in PNN density in either the top (C) or bottom (D) layer analysis. A-B. In stark contrast, going through the pup gathering paradigm causes many different alterations in PNN density between surrogates, as well as within genotype which are pervasive in the top (A) and bottom (B) layers. A-B. (IV-I) After the pup gathering paradigm, PNN density is increased in the right hemisphere of S1FL, in both the top and bottom layers. This alteration is not seen in the Het, suggesting PNN increase in this region is important for successful behavior. (II-I) While there are no differences between NW and NH in the whole subregion analysis, in the top layer (A) there is increased PNN density in the right hemisphere of S1ULp in NH, which is present after behavior as well (A, III). In the bottom layers (B), PNN density is increased in the S1FL right hemisphere, however this difference is no longer present after behavior. (III-IV/II) PNN density in SH within the S1BF is increased in comparison to SW and NH, in both hemispheres and both layer segments; suggesting tactile perception through the whiskers is important for this behavior. In the top layers, SH has increased PNN density in the left hemisphere of S1DZ when compared to NH and SW. (III-IV) After behavior, PNNs are increased in the right S1ULp in both the top and bottom layers (A-B). In the bottom layers, the S1FL increases in the left hemisphere. Many increases in PNN density in SH (III) as compared to SW (IV) and NH (II) are pervasive in both layer segments; left and right S1BF and right S1ULp.

6 WO PNN Layer Summary

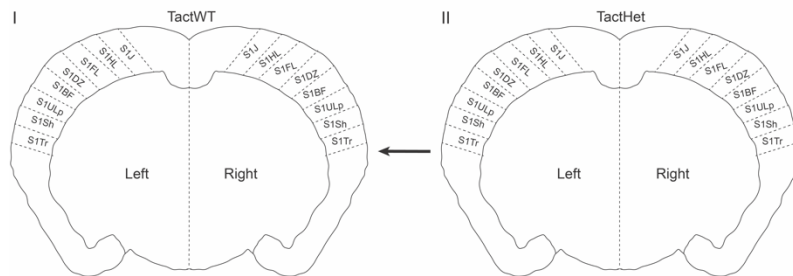
A Maternal Top Layer



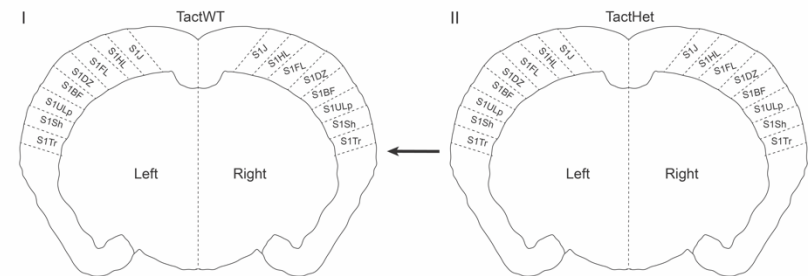
B Maternal Bottom Layer



C Tactile Top Layer



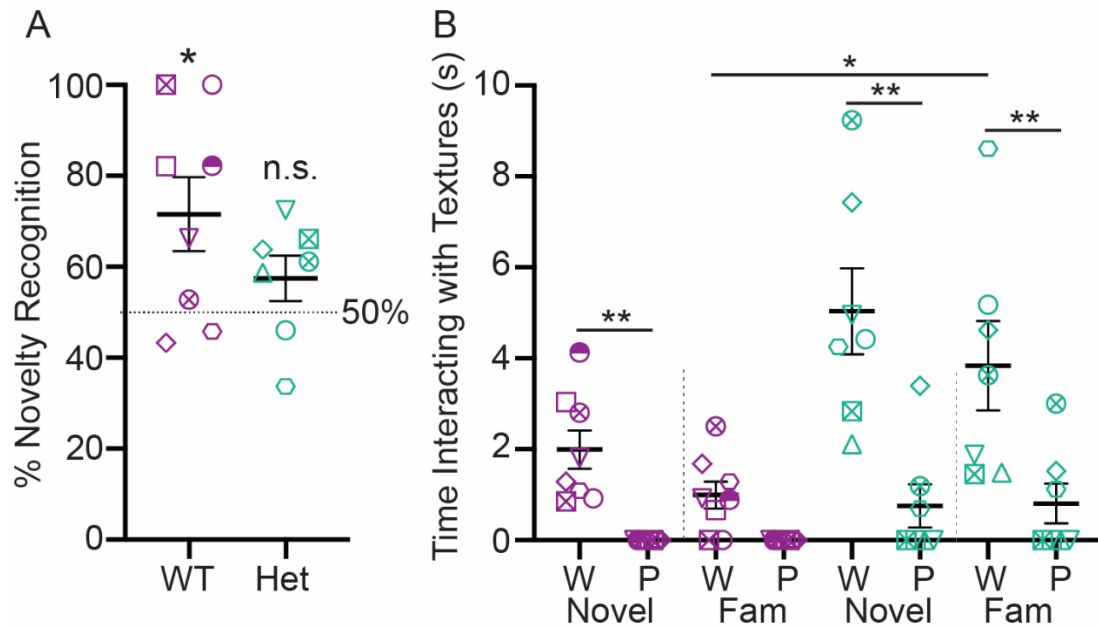
D Tactile Bottom Layer



Supplementary Table 3.1. Number of animals included in each simple tactile behavior analysis and the reasons for exclusion from analysis.

On texture discrimination day 1, the 12 WO and one 6 WO TacHet were excluded due to video lagging/skipping during investigation interactions making the onset and offset of investigation hard to time stamp. The second 6 WO TacHet had an exploration time under 2 seconds. The 12 and 6 WO TacWT did not interact with either texture. On texture discrimination day 2, the 12 and 6 WO TacWT was excluded due to interaction time under 2 seconds. No animals were excluded from the novel object recognition or adhesive tests.

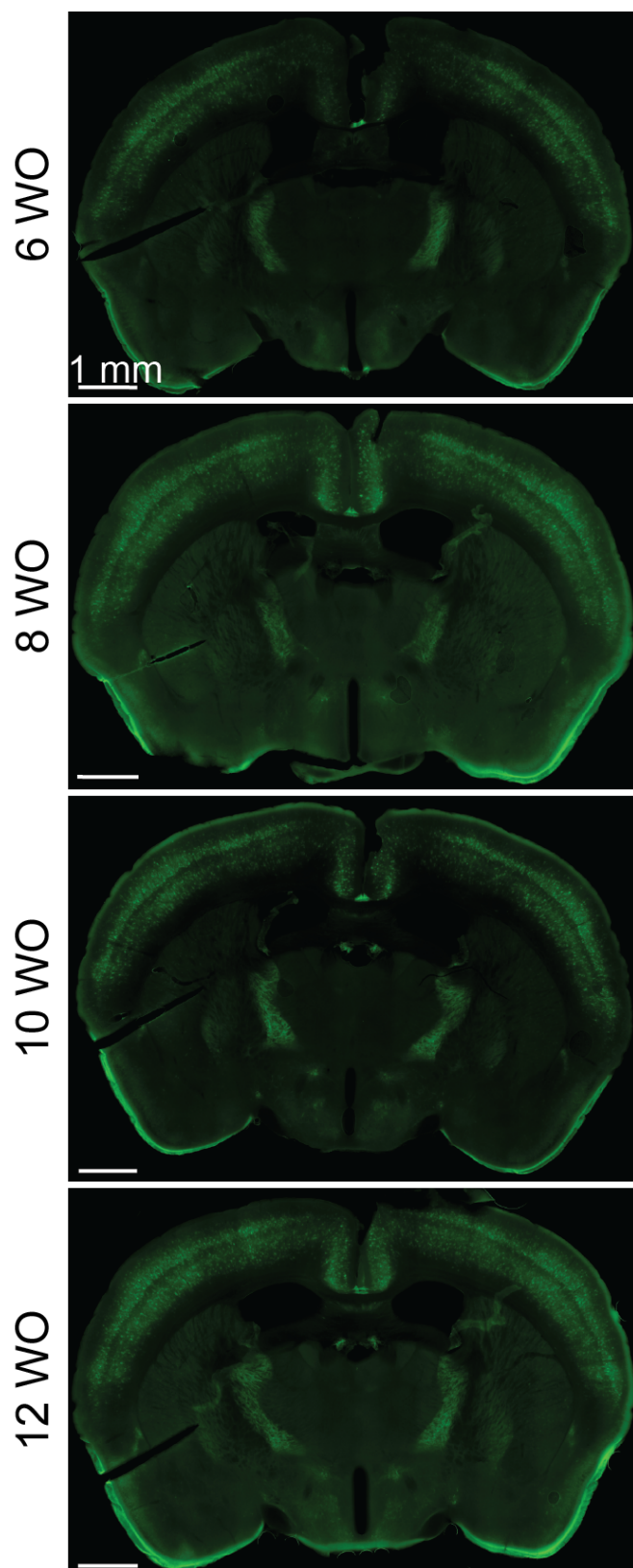
		Included in Analysis		Excluded from Analysis	
		WT	Het	WT	Het
12 WO	Texture Discrimination Day 1	8	7	1	1
	Texture Discrimination Day 2	8	8	1	0
	Novel Object Recognition	9	8	0	0
	Adhesive Test	9	8	0	0
6 WO	Texture Discrimination Day 1	9	7	1	2
	Texture Discrimination Day 2	9	9	1	0
	Novel Object Recognition	10	9	0	0
	Adhesive Test	10	9	0	0

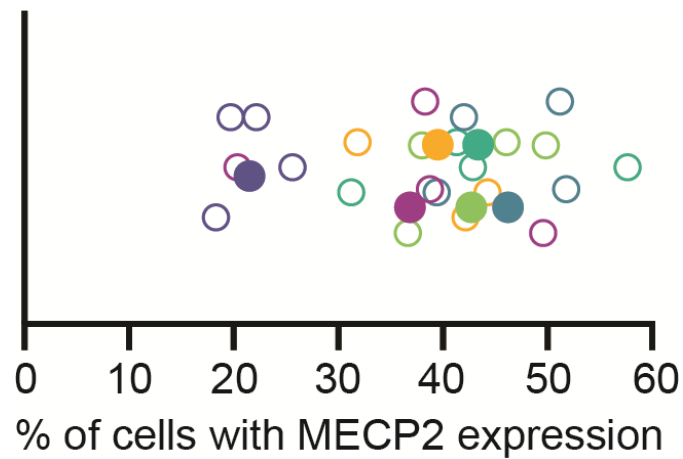


Supplementary Figure 3.1. 12 WO TacWTs show novelty recognition in the first three minutes of the texture discrimination assay on day 1.

The first three minutes of the ten-minute testing phase were analyzed to correct for loss of novelty. A. 12 WO TacWT (purple) show a significant novelty preference whereas the TacHet (green) still do not. B. Within the first three minutes, 12 WO TacHets still use both whiskers and forepaws to interact with the textures, however no climbing occurs in the first three minutes. TacWT exclusively use their whiskers. A. One sample t-test, 50% chance n.s.=not significant, * $p < 0.05$. B. *Kruskal-Wallis* followed by *uncorrected Dunn's test*, * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure 3.2. Representative NaïveWT sections from each age-group show variation in PNN density when imaged at the same exposure setting. Comparative epifluorescent images of PNN expression at 6-, 8-, 10- and 12-week-old WT brains showing that high-intensity PNN expression differs between age groups when imaged at the same exposure setting. In the time course assay, age groups and cohorts were imaged individually based on the Het of that cohort; so we cannot directly compare between age-groups. There is variation, with 8 WO females appearing to have higher PNN densities than the other age groups in Table 3.1. This variation appears to be present when representative sections from each age group are imaged at the same setting.





Supplementary Figure 3.3. Variation in MECP2 expression per animal in the 6 WO SurHets.

Four images were analyzed per SH to obtain the percentage of MECP2+/DAPI+ cells within the SS1 to correlate MECP2 expression to behavioral performance in Figure 3.6. Here the filled circles show the average percentage of MECP2+ cells in the SS1 of each brain (indicated by the different colors). The hollow circles show the percentage of MECP2+/DAPI+ cells per image. There does not appear to be large variations between values within animal. N = 6 animals, 4 images per animal.

CONCLUSION

Our general approach to dissecting Rett Syndrome (RTT) pathogenesis is to establish a concise cellular phenotype, within a relevant behavioral circuit, in the *Mecp2*-heterozygous (Het) female mouse model. We have hypothesized that MECP2 regulates experience-dependent plasticity during periods of heightened sensory and social experiences. Previously, we showed that the dysregulation of the inhibitory cortical network during an experience-dependent event is a common motif in RTT pathogenesis in the developing *Mecp2*-null visual cortex (heightened sensory experience) and adult Het auditory cortex (social experience) (Krishnan et al., 2015, 2017). In both contexts, perineuronal nets (PNNs), markers of restricted plasticity, were atypically or precociously expressed in the RTT model cortex when compared to the WT. These deficits in visual and auditory processing support the emerging hypothesis that RTT is a sensory processing disorder, as sensory deficits precede the onset of RTT characteristic symptoms in female patients. In this dissertation, we identified a new sensory modality, tactile sensory processing, that has deficits in experience-dependent plasticity in the Het primary somatosensory cortex (SS1) in an age-dependent and context-dependent manner.

In chapter 1, we discussed the exploratory PNN expression analysis that led to the identification of the SS1 as a region that shows atypical expression of PNNs in the surrogate (Sur) Het, similar to the auditory cortex. Due to the important role of somatosensation in the pup retrieval paradigm (Beach and Jaynes, 1956; Stern, 1990), known connectivity between the SS1 and auditory cortex in social whisking (Rao et al., 2014), and the role of the SS1 in tactile processing (Brecht, 2007, 2017), we chose to analyze SS1 PNN expression in more detail, as described in chapter 2. In chapter 2, we present a detailed analysis of hemisphere- and subregion-specific alterations in PNN density that occur before and after pup retrieval in the adult SS1. In chapter 3, we explored the behavioral implications of the adult Het having atypical increase of PNN density before the pup retrieval paradigm, which led to the identification of tactile processing deficits in the adult Het. We reported a developmental shift in PNN expression within the NaïveHet SS1 between the 6 and 12 WO time points, then found

that the 6 WO Hets did not show whisker-based tactile processing deficits in the simple tactile assays. However, 6 WO Hets were inefficient at the pup gathering behavior, showing that MECP2 deficiency affects experience-dependent plasticity in complex multisensory but not simple tactile contexts, in an age-dependent manner.

Remodeling of SS1 PNNs within the adult Mecp2^{WT} after surrogacy

In chapter two, we characterized PNN density changes that occurred in the adult Het and WT before and after the pup gathering paradigm in the SS1. While we were expecting PNN density alterations in the SurHet (based on data from chapter 1), we also found dynamic PNN density changes in the SurWT, similar to what was seen in the motor cortex in chapter 1 (Figure 4 A). PNNs are stable structures that form around PV+ interneurons after a period of heightened plasticity during development and adulthood, solidifying new synaptic connections that have formed (Miyata and Kitagawa, 2017; Sorg et al., 2016). We found increased PNN density within the SurWT right S1BF (barrel field) when compared to the NaïveWT, as well as increased PNN densities in the right S1FL and S1DZ when compared to the left hemisphere of the SurWT (Figure 4, A I-IV). Together, these data suggest that PNN formation in these subregions is important for efficient pup retrieval. Additionally, we found a reduction in PNN density in the left S1FL of the SurWT when compared to NaïveWT, showing that dynamic remodeling of PNNs is also important in the execution of the pup retrieval paradigm.

The adult SurWT data suggests that there are pup gathering experience-specific circuits within the SS1 that undergo alterations in PNN density for efficient pup gathering behavior. We can also compare this to PNN density changes that are occurring in the SurHet SS1, to see if the SurHet is going through similar or different plasticity changes (Figure 4, A). The SurHet also has increased PNN densities in the right S1FL and S1DZ when compared to the left hemisphere, however there is no increase in the right S1BF like in the SurWT (Figure 4, A II-III). This could be due to the already increased PNN density in the S1BF of the NaïveHet when compared to the NaïveWT (Figure 4, A I-II). As an increase in PNN density is supposed to occur after

the behavior based on SurWT results (Figure 4, A IV) the increase in the NaïveHet could be preventing any experience-dependent plasticity that is supposed to happen in the S1BF from occurring.

MECP2 deficiency causes developmental shift in SS1 PNN density

The adult Het had increased PNN density when compared to the WT in the SS1 before going through the behavior, suggesting that the adult Hets may have tactile sensory deficits that contribute to the inefficient pup retrieval phenotype due to restricted plasticity in the region (Figure 4, A I-II). To identify the origin of this PNN density shift in the NaïveHet SS1, we did a time course analysis of SS1 PNN density in the NaïveWT and NaïveHet brain, as there have been some descriptive studies that characterize temporal changes in PNN density within the SS1, but not at this specific age (Ueno et al., 2018, 2019). We found there to be no differential PNN density in the SS1 between NaïveWT and NaïveHet at 6 weeks of age (WO) (Figure 4, B I-II).

Currently, we do not know the cause of this shift in PNN density between 6 WO and 12 WO, as most SS1 critical periods occur within the first 14 days of life (Erzurumlu and Gaspar, 2012). One explanation is that there could be morphological deficits in the S1BF neurons that are causing this developmental shift. MECP2 is important for the maturation, maintenance, and function of neurons (Bedogni et al., 2016; McGraw et al., 2011; Nguyen et al., 2012). Neurons in the SS1 may be particularly sensitive to MECP2 deficiency between the 6-12 WO time points, which was an MECP2 expression sensitive time point found in the MECP2 conditional knockout studies (in males) (Cheval et al., 2012; Du et al., 2016). In the *Mecp2*-null male, neurons showed a reduction in dendritic length and spine density in the motor and barrel field cortices (Belichenko et al., 2009; Lee et al., 2017). This correlated with decreased SS1 area and decreased activity in the region (Lee et al., 2017; Moroto et al., 2013). MECP2-negative cortical neurons in the Het have similar morphological changes, though these studies were done in the motor cortex of 6-month-old Hets (Belichenko et al., 2009; Rietveld et al., 2015).

These studies suggest that there may be neurons with atypical morphology in the Het S1BF that are influencing this developmental shift in PNN density. To test this, an in-depth analysis of neuronal morphology would have to be done in the Het SS1 at multiple developmental time points. Atypical neuron morphology could affect signaling in the somatosensory cortex in multiple different ways. For example (based on known SS1 connectivity), underdeveloped thalamocortical neurons would limit the amount of tactile input entering the SS1, underdeveloped excitatory neurons in layer IV could prevent the relaying of tactile input to layers II & III, lessening the effect of SS1 modulation on other sensory cortices (Cohen et al., 2011a; Petersen, 2007; Rao et al., 2014). Regardless, the developmental shift in PNN density within the SS1 suggests that MECP2 deficiency is regulating the expression and formation of PNNs in an age-dependent manner.

Mecp2^{Het} show age-dependent tactile perception deficits

Based on the increased PNN density in the 12 WO (adult) NaïveHet SS1, indicating restricted plasticity, we speculated that these animals would present with whisker-based tactile perception deficits. If the atypical PNN density increase were causing tactile deficits, then the 6 WO NaïveHet would not present with these deficits, as they don't have atypical PNN density in the SS1. In the texture discrimination assays, we noticed that the 12 WO TacHet spent significantly more time exploring the textures and used compensatory investigation mechanisms, like investigating with the forepaws and climbing, when compared to the 12 WO TacWT, which interact with solely their whiskers (Figure 5, row 3 blue). In contrast, the 6 WO TacHet, and TacWT, perform similarly to the 12 WO TacWT (Figure 5, row 3). Animals that are unable to receive tactile input from the whiskers to the SS1 (via barrel cortex lesioning) eventually use their forepaws to investigate the textures (Guic-Robles et al., 1992). The 12 WO TacHets use of forepaws and climbing to interact with the textures suggests that they are unable to process the texture information from their whiskers. This could be tested by removing the whiskers from the 12 WO TacWT before the battery of simple tactile tasks, to see if they will use their forepaws to interact with the textures, which is what

should happen based on previous literature (Guic-Robles et al., 1992; Orefice et al., 2016; Wu et al., 2013).

The analysis of this behavior was done by manual coding of each 10-minute testing phase, while the researchers were blinded to the genotype and age of the mouse. After this manual analysis, we noted slight differences in the behavior of each animal, such as hyperactivity or lack thereof on the periphery of the testing cage, approaching textures nose or whisker first, and approaching textures consistently with either the right or left whisker bundle. These individual differences are also reflected in the novelty recognition data from chapter 3, as there was individual variability between animals that presented with a novelty or familiarity preference. In novel recognition tasks, like the texture discrimination assay, the default is to assume that, if they can sense the difference, all animals will prefer to spend time with the novel texture/object over the familiar one (Antunes and Biala, 2012; Ennaceur, 2010). However, a familiarity preference still shows that the animal can tell the difference between the two textures/objects, they just spend more time with the familiar texture/object (Ennaceur, 2010; Ennaceur et al., 2005). We see this split in our dataset. For future studies and analysis, we suggest either separating the two populations (above and below 50% novelty) to see if there is a significant novelty or familiarity preference in those subgroups or evaluating each animal individually. We also recommend further analysis of this behavior in an automated tracking software to expand upon the observations made by the manual behavior coders over the 10-minute behavior.

The behavioral difference between the 12 WO TacHet and 12 WO TacWT suggests that the atypical increase of PNNs in the 12 WO NaïveHet is causing or is indicative of a dysregulated inhibitory network that is causing, tactile deficits in the adult Het. This is supported by the 6 WO Tac findings, as they do not have atypical PNN density in Naïve state and show no deficits in tactile perception (Figure 5). Pup related tactile input from the perioral area (whiskers, jaw, snout) is important for the onset of learned maternal behaviors, like the pup retrieval assay (Kenyon et al., 1981; Morgan et al., 1992; Stern and Kolunje, 1989). If increased PNN density in the 12 WO NaïveHet

is restricting the processing of tactile feedback, it could be contributing to the SurHet inefficient pup retrieval. This line of thought suggests that the 6 WO SurHet would perform efficiently at the retrieval behavior.

Future studies should degrade PNNs in the 12 WO Het, via chondroitinase ABC injection, before the pup retrieval and simple tactile assay. If PNNs were degraded, we speculate the 12 WO Het would show no tactile deficits and have a partial rescue of the pup gathering behavior. If there was a partial rescue of behavior, dual PNN removal from the adult Het auditory cortex and SS1 should be done, as we have already shown the importance of the auditory cortex in this behavior (Krishnan et al., 2017).

SurHets are inefficient at pup retrieval but have age-dependent alterations in PNN density

Despite having no atypical expression in PNN density before the behavior, the 6 WO SurHet (Figure 5, row 2 green) is inefficient at the pup retrieval paradigm, just like the adult SurHet (blue) supporting our overall hypothesis that MECP2 regulates experience-dependent plasticity during social experiences, like the pup retrieval assay. Based on this we thought that the 12 and 6 WO SurHet would show similar dysregulation of PNN expression in the SS1. After whole brain manual quantification of high-intensity PNN density across the SS1, we found that the 6 WO SurHet had significantly higher PNN density than the NaïveHet and SurWT. However, when the analysis was split by subregion and hemisphere, we noticed a different pattern of PNN density alterations between the 6 WO and 12 WO SurHets (Figure 4).

The starkest difference between the 12 and 6 WO SurHet is that there were no PNN density differences between the 12 WO SurWT and SurHet (Figure 4 A III & IV). The 6 WO SurHet has significantly higher PNN density in the S1BF, right S1DZ and left S1ULp when compared to the 6 WO SurWT (Figure 4 B III-IV). There is a hemisphere bias of PNN density in the S1DZ, but the 6 WO is higher in the left hemisphere whereas the 12 WO is higher in the right hemisphere (Figure 4 A & B III, arrows). The 6 WO SurHet has a significant PNN density increase in the S1BF when compared to the NaïveHet (Figure 4 B II-III), which is not seen at 12 WO (Figure 4 A

II-III), but this could be due to the 12 WO NaïveHet already having a significant increase in that subregion. These differences in PNN expression pattern between the two SurHet time points further shows that MECP2 deficiency is dysregulating experience-dependent plasticity mechanisms, like PNN formation, in an age-dependent manner.

MECP2 deficiency affects experience-dependent plasticity mechanisms in context-dependent manner at 6 WO

6 WO NaïveHet do not have atypical expression of PNNs in the SS1, indicating that there are no restrictions to plasticity before going through the pup retrieval or simple tactile behavior tasks. We report that the 6 WO Het shows no behavioral deficits in the simple tactile assays, but is inefficient at the complex, multisensory pup retrieval assay (Figure 5 column 4). Based on our hypothesis about MECP2, we expected alterations in PNN density in the SurHet and not TacHet SS1. After the pup gathering behavior the SurHet showed significant PNN density increases in the whole brain (Figure 6, A), split hemisphere (Figure 4, B), and top layer and bottom layer analyses (Figure 3.12 A & B). The TacHet showed very subtle changes in PNN density after behavior which were present in the whole brain analysis (Figure 6, B) but did not hold up in the split hemisphere or top and bottom layer analysis (Figure 3.12, C & D). Together, the 6 WO data shows that MECP2 deficiency affects the regulation experience-dependent plasticity, and PNN expression, in a complex multisensory social context and not in simple tactile context. But why is a difference seen between the social and asocial task?

A characteristic of many neurodevelopmental disorders is a shift in the cortical excitatory/inhibitory balance; in the RTT mouse models, this shift favors inhibition (Dani et al., 2005; Gogolla et al., 2014). MECP2 is an activity-dependent gene expression regulator which directly binds to the promoter regions of genes that make key inhibitory signaling molecules, GAD67 and PV (Cohen et al., 2011b). The precocious and increased expression of these molecules after heightened sensory or social experience, restricting experience-dependent plasticity, is a common cellular

phenotype seen in MECP2-deficient mice (Krishnan et al., 2015, 2017; Morello et al., 2018).

The pup retrieval behavior involves the multisensory integration of pup cues for proper execution of the behavior. There is a large amount of sensory information coming into the brain during the multisensory social paradigm (auditory, olfactory, tactile) in contrast to the simple tactile paradigm (tactile). Social whisking assays have shown that social whisking induces stronger membrane potential fluctuations in layer II & III pyramidal neurons (Lenschow and Brecht, 2015) and increased firing rate in layer V projection neurons (Bobrov et al., 2014) when compared to active whisker investigation of an object. Plasticity in the SS1 is regulated by inhibitory cortical networks as increased excitatory input to the S1BF, via increased whisker stimulation, is modulated by an increase in GABAergic synapses (Knott et al., 2002). From these studies, we can speculate that during the pup retrieval assay, in which the surrogates have six days of constant social interaction with the pups and other adults, the SS1 is receiving more excitatory input than in the simple asocial tactile behavior. Additionally, the surrogates are integrating auditory and olfactory sensory cues which have been shown to induce plasticity in the auditory cortex (Cohen et al., 2011a), plasticity that was dysregulated in the adult SurHet (Krishnan et al., 2017). From our 6 WO behavioral phenotype and what is known about MECP2 function, we can speculate that the increased stimulation from the complex social paradigm is triggering the dysregulation of the inhibitory cortical network in the 6 WO SurHet. To address this, future studies could quantify the expression of PV and GAD67 in the SS1, similar to Krishnan et al., 2015, 2017.

The pup retrieval assay is just a small component of learned maternal behavior, as the surrogates are co-housed with the mother and pups, they are getting constant sensory stimulation from multiple individuals, constantly. Since we have shown that there is a behavioral phenotype in this complex multisensory social assay, and not one in the simple tactile behavior, we hypothesize that the 6 WO Het would still show experience-dependent plasticity deficits when tested in a simpler multisensory

paradigm. A follow-up simple multisensory assay would be the gap test, where there is only one other animal – typically an adult – to interact with for short periods of time (Bobrov et al., 2014; Rao et al., 2014). An alternative could be the three-chamber sociability (one mouse and one object) and social novelty (two mice) tasks, which are often used to study social deficits in neurodevelopmental rodent models (Orefice et al., 2016; Rein et al., 2020).

Summary

Within the SS1, I have identified three different contexts through which we can further study MECP2's role in regulating experience-dependent plasticity, as measured here by PNN expression, at the molecular level. The first, is through the developmental shift in PNN density between the 6- and 12-week-old NaïveHet when compared to the NaïveWT. The second, is through simple tactile assays between the two age groups, where the 12 WO TacHet shows deficits in tactile sensory processing, but the 6 WO TacHet does not. The third is how MECP2 deficiency affects experience-dependent plasticity during complex multisensory, not simple tactile, behaviors in the 6 WO females. This identified an additional sensory modality that has deficits in the 'pre-symptomatic' female Het mouse model. Further supporting the emerging hypothesis that RTT is a sensory processing disorder, which gives rise to characteristic syndromic symptoms.

References

- Antunes, M., and Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process* 13, 93–110.
- Beach, F.A., and Jaynes, J. (1956). Studies of Maternal Retrieving in Rats. III. Sensory Cues Involved in the Lactating Female's Response To Her Young. *Behaviour* 10, 104–124.
- Bedogni, F., Cobolli Gigli, C., Pozzi, D., Rossi, R.L., Scaramuzza, L., Rossetti, G., Pagani, M., Kilstrup-Nielsen, C., Matteoli, M., and Landsberger, N. (2016). Defects During *Mecp2* Null Embryonic Cortex Development Precede the Onset of Overt Neurological Symptoms. *Cereb. Cortex* 26, 2517–2529.

Belichenko, N.P., Belichenko, P.V., and Mobley, W.C. (2009). Evidence for both neuronal cell autonomous and nonautonomous effects of methyl-CpG-binding protein 2 in the cerebral cortex of female mice with *Mecp2* mutation. *Neurobiology of Disease* 34, 71–77.

Bobrov, E., Wolfe, J., Rao, R.P., and Brecht, M. (2014). The Representation of Social Facial Touch in Rat Barrel Cortex. *Current Biology* 24, 109–115.

Brecht, M. (2007). Barrel cortex and whisker-mediated behaviors. *Curr. Opin. Neurobiol.* 17, 408–416.

Brecht, M. (2017). The Body Model Theory of Somatosensory Cortex. *Neuron* 94, 985–992.

Cheval, H., Guy, J., Merusi, C., De Sousa, D., Selfridge, J., and Bird, A. (2012). Postnatal inactivation reveals enhanced requirement for MeCP2 at distinct age windows. *Human Molecular Genetics* 21, 3806–3814.

Cohen, L., Rothschild, G., and Mizrahi, A. (2011a). Multisensory integration of natural odors and sounds in the auditory cortex. *Neuron* 72, 357–369.

Cohen, S., Gabel, H.W., Hemberg, M., Hutchinson, A.N., Sadacca, L.A., Ebert, D.H., Harmin, D.A., Greenberg, R.S., Verdine, V.K., Zhou, Z., et al. (2011b). Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. *Neuron* 72, 72–85.

Dani, V.S., Chang, Q., Maffei, A., Turrigiano, G.G., Jaenisch, R., and Nelson, S.B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 102, 12560–12565.

Du, F., Nguyen, M.V.C., Karten, A., Felice, C.A., Mandel, G., and Ballas, N. (2016). Acute and crucial requirement for MeCP2 function upon transition from early to late adult stages of brain maturation. *Hum. Mol. Genet.* 25, 1690–1702.

Ennaceur, A. (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behavioural Brain Research* 215, 244–254.

Ennaceur, A., Michalikova, S., Bradford, A., and Ahmed, S. (2005). Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. *Behavioural Brain Research* 159, 247–266.

Erzurumlu, R.S., and Gaspar, P. (2012). Development and critical period plasticity of the barrel cortex: Barrel cortex plasticity. *European Journal of Neuroscience* 35, 1540–1553.

Gogolla, N., Takesian, A.E., Feng, G., Fagiolini, M., and Hensch, T.K. (2014). Sensory Integration in Mouse Insular Cortex Reflects GABA Circuit Maturation. *Neuron* 83, 894–905.

Guic-Robles, E., Jenkins, W.M., and Bravo, H. (1992). Vibrissal roughness discrimination is barrelcortex-dependent. *Behavioural Brain Research* 48, 145–152.

Kenyon, P., Cronin, P., and Keeble, S. (1981). Disruption of maternal retrieving by perioral anesthesia. *Physiology & Behavior* 27, 313–321.

Knott, G.W., Quairiaux, C., Genoud, C., and Welker, E. (2002). Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. *Neuron* 34, 265–273.

Krishnan, K., Wang, B.-S., Lu, J., Wang, L., Maffei, A., Cang, J., and Huang, Z.J. (2015). MeCP2 regulates the timing of critical period plasticity that shapes functional connectivity in primary visual cortex. *Proc. Natl. Acad. Sci. U.S.A.* 112, E4782-4791.

Krishnan, K., Lau, B.Y.B., Ewall, G., Huang, Z.J., and Shea, S.D. (2017). MECP2 regulates cortical plasticity underlying a learned behaviour in adult female mice. *Nature Communications* 8, 14077.

Lee, L.-J., Tsytsarev, V., and Erzurumlu, R.S. (2017). Structural and functional differences in the barrel cortex of *Mecp2* null mice. *Journal of Comparative Neurology* 525, 3951–3961.

Lenschow, C., and Brecht, M. (2015). Barrel Cortex Membrane Potential Dynamics in Social Touch. *Neuron* 85, 718–725.

McGraw, C.M., Samaco, R.C., and Zoghbi, H.Y. (2011). Adult Neural Function Requires MeCP2. *Science* 333, 186–186.

Miyata, S., and Kitagawa, H. (2017). Formation and remodeling of the brain extracellular matrix in neural plasticity: Roles of chondroitin sulfate and hyaluronan. *Biochimica et Biophysica Acta* 1861, 2420–2434.

Morello, N., Schina, R., Pilotto, F., Phillips, M., Melani, R., Plicato, O., Pizzorusso, T., Pozzo-Miller, L., and Giustetto, M. (2018). Loss of *Mecp2* Causes Atypical Synaptic and Molecular Plasticity of Parvalbumin-Expressing Interneurons Reflecting Rett Syndrome–Like Sensorimotor Defects. *Eneuro* 5, ENEURO.0086-18.2018.

Morgan, H.D., Fleming, A.S., and Stern, J.M. (1992). Somatosensory control of the onset and retention of maternal responsiveness in primiparous Sprague-Dawley rats. *Physiology & Behavior* 51, 549–555.

Moroto, M., Nishimura, A., Morimoto, M., Isoda, K., Morita, T., Yoshida, M., Morioka, S., Tozawa, T., Hasegawa, T., Chiyonobu, T., et al. (2013). Altered somatosensory barrel cortex refinement in the developing brain of *Mecp2*-null mice. *Brain Research* 1537, 319–326.

Nguyen, M.V.C., Du, F., Felice, C.A., Shan, X., Nigam, A., Mandel, G., Robinson, J.K., and Ballas, N. (2012). MeCP2 Is Critical for Maintaining Mature Neuronal Networks and Global Brain Anatomy during Late Stages of Postnatal Brain Development and in the Mature Adult Brain. *Journal of Neuroscience* 32, 10021–10034.

Orefice, L.L., Zimmerman, A.L., Chirila, A.M., Sleboda, S.J., Head, J.P., and Ginty, D.D. (2016). Peripheral Mechanosensory Neuron Dysfunction Underlies Tactile and Behavioral Deficits in Mouse Models of ASDs. *Cell* 166, 299–313.

Petersen, C.C.H. (2007). The Functional Organization of the Barrel Cortex. *Neuron* 56, 339–355.

Rao, R.P., Mielke, F., Bobrov, E., and Brecht, M. (2014). Vocalization–whisking coordination and multisensory integration of social signals in rat auditory cortex. *ELife* 3.

Rein, B., Ma, K., and Yan, Z. (2020). A standardized social preference protocol for measuring social deficits in mouse models of autism. *Nat Protoc* 15, 3464–3477.

Rietveld, L., Stuss, D.P., McPhee, D., and Delaney, K.R. (2015). Genotype-specific effects of *Mecp2* loss-of-function on morphology of Layer V pyramidal neurons in heterozygous female Rett syndrome model mice. *Frontiers in Cellular Neuroscience* 9.

Sorg, B.A., Berretta, S., Blacktop, J.M., Fawcett, J.W., Kitagawa, H., Kwok, J.C.F., and Miquel, M. (2016). Casting a Wide Net: Role of Perineuronal Nets in Neural Plasticity. *The Journal of Neuroscience* 36, 11459–11468.

Stern, J.M. (1990). Multisensory regulation of maternal behavior and masculine sexual behavior: A revised view. *Neuroscience & Biobehavioral Reviews* 14, 183–200.

Stern, J.M., and Kolunie, J.M. (1989). Perioral anesthesia disrupts maternal behavior during early lactation in Long-Evans rats. *Behav. Neural Biol.* 52, 20–38.

Ueno, H., Takao, K., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Okamoto, M., Aoki, S., and Ishihara, T. (2018). Age-dependent and region-specific alteration of parvalbumin neurons and perineuronal nets in the mouse cerebral cortex. *Neurochemistry International* 112, 59–70.

Ueno, H., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Matsumoto, Y., Okamoto, M., and Ishihara, T. (2019). Layer-specific expression of extracellular matrix molecules in the mouse somatosensory and piriform cortices. *IBRO Reports* 6, 1–17.

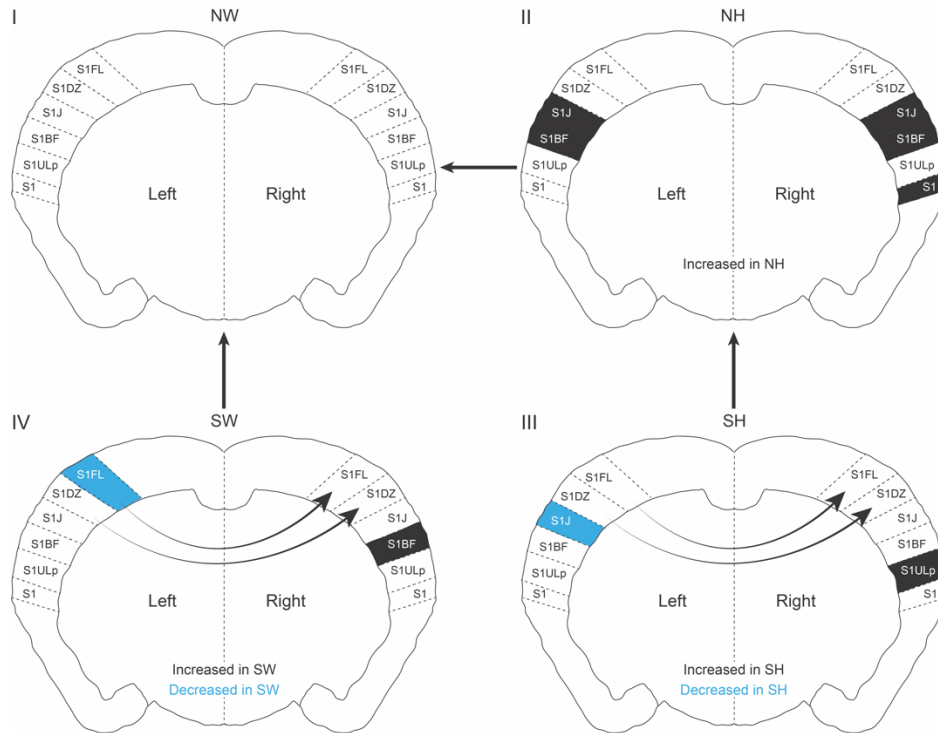
Wu, H.-P.P., Ioffe, J.C., Iverson, M.M., Boon, J.M., and Dyck, R.H. (2013). Novel, whisker-dependent texture discrimination task for mice. *Behav. Brain Res.* 237, 238–242.

Appendix

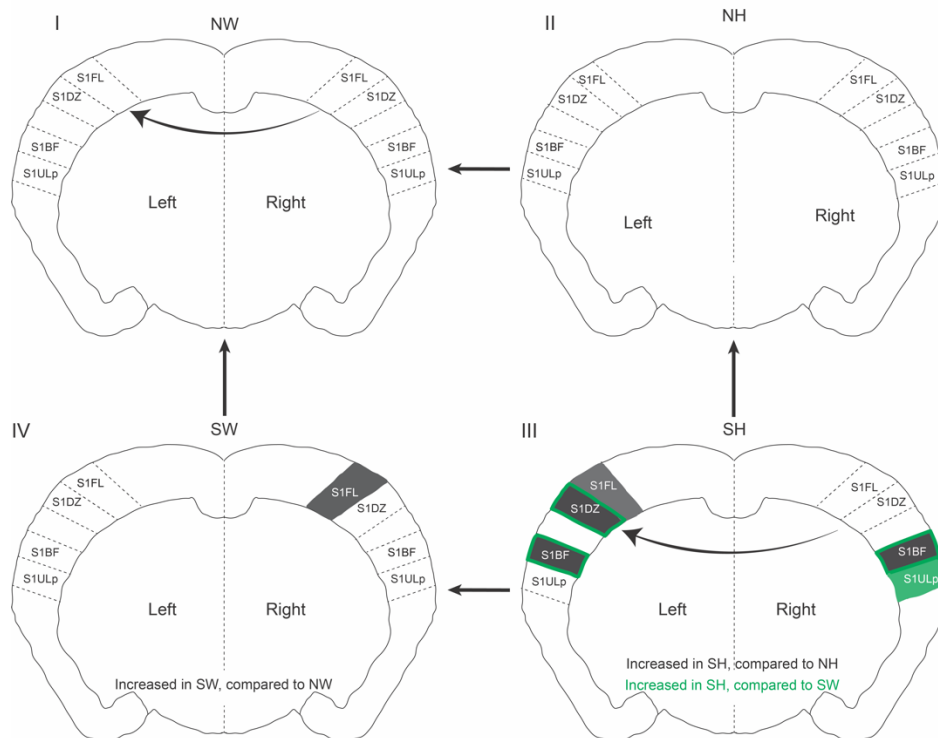
Figure 4. Summary of the age-dependent alterations in PNN density before and after pup gathering behavior in a subregion- and hemisphere-specific manner.

A. For 12 WO timepoint, significant increases in PNN density are marked in black while significant reductions are marked in blue. B. For 12 WO timepoint, significant increases within condition are marked in green while significant increases between genotype are marked in grey and black (the darker the color, the more significant the PNN density change). A-B. Arrows indicate left-right asymmetry within brain, arrowhead indicates the higher PNN density. III-IV. A. There were no significant alterations in PNN density between the 12 WO SW and SH. B. The 6 WO SH had increased PNN density in the S1BF, left S1DZ and right S1ULp when compared with SW. II-III. A. 12 WO SH showed PNN density increase in the right S1ULp but a reduction in PNN density in the left S1J when compared to NH. B. 6 WO SH had a PNN density increase in the S1BF, right S1DZ and S1FL when compared to NH. There was no reduction of PNN density seen in the SH. I-II. A. 12 WO NH had increased PNN density in S1BF and S1J, with no hemisphere-specificity, and increase in the right S1, when compared to NW. B. There were no differences in PNN density between the 6 WO NH and NW. I-IV. A. The adult SW underwent dynamic alterations in PNN density with an increase in the right S1BF and reduction in left S1FL when compared to NW as well as asymmetrical increase of PNNs in the right S1FL and S1DZ. B. In contrast, the 6 WO SW only showed a slight increase in PNN density in the right S1FL. A. Modified from Figure 2.15, describing data from Table 2.1. B. Describing PNN density data from Figure 3.8.

A. 12 WO Pup Gathering Summary



B. 6 WO Pup Gathering Summary



		12 WO Het	6 WO Het
Pup Retrieval Assay 	Latency	+	+
	Error	+	+
Texture Discrimination 	Novelty %	=	=
	Investigation (s)	+	=
	Compensatory Investigation	w p c	=
Novel Object Recognition 	Novelty %	=	=
	Investigation (s)	=	=
	Compensatory Investigation	=	=
Adhesive Test 	Removal Latency	=	=
	Latency to First Attempt	=	=
	Errors	=	=

Figure 5. Summary of the Het behavioral performance in the complex and simple paradigms, when compared to WT.

Column 1 shows the behavior, column 2 has the behavioral readout, columns 3 (12 WO) and 4 (6 WO) show the significant ('+' and highlighted) or not significant (=) behavioral difference between the Het and WT in that behavior. Row 2 shows that the 12 and 6 WO SurHet did not perform the pup retrieval behavior efficiently when compared to SurWT. Row 3 shows that the 12 WO TacHet spends significantly more time investigating textures and investigates textures with its forepaws and through climbing, while the TacWT uses only whiskers. 6 WO TacHet and TacWT perform similarly, both utilizing their whiskers during investigation. Rows 4 & 5 show that all of the animals perform similarly in these tasks.

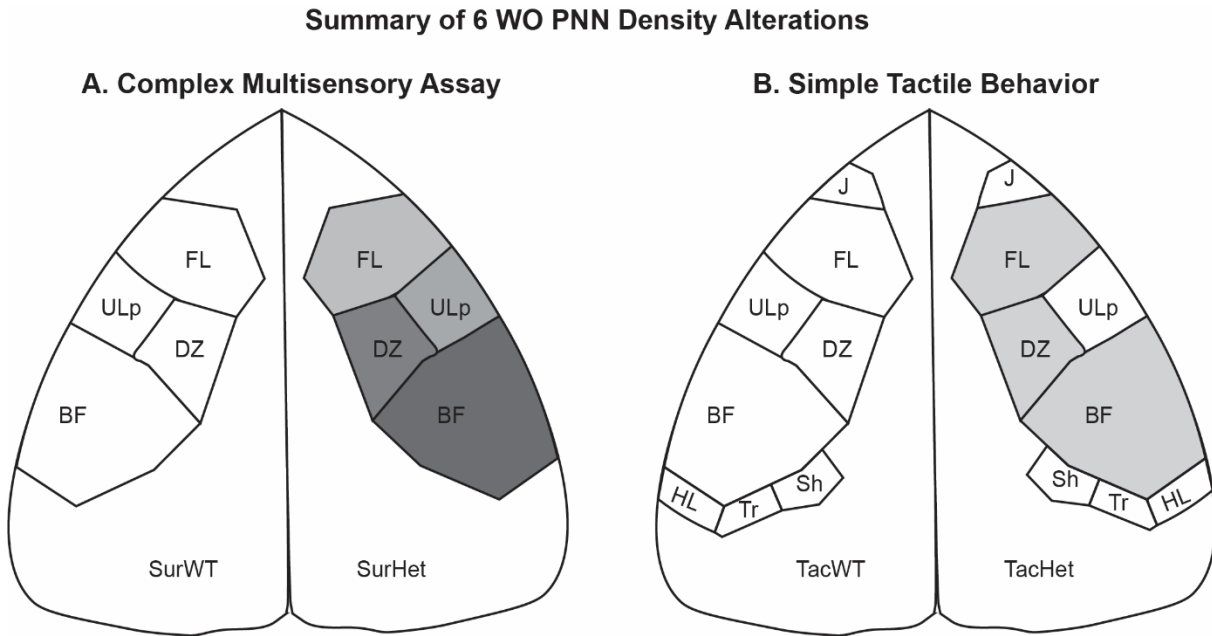


Figure 6. Summary of whole subregion changes in Het PNN density after behavior in the complex multisensory and simple tactile paradigm.

A. The SurHet had significantly increased PNN density in the S1FL, S1ULp, S1DZ and S1BF (as marked by the progressively darker gray shading) when compared to the SurWT. The significant increase of PNN density in the SurHet when compared to the SurWT was much larger than the increase between TacHet and TacWT. B. When that was broken into subregions, the TacHet (B) showed subtle increases in PNN density in the S1BF, S1DZ, and S1FL. However, these changes were not present when the hemispheres were split. Gray-filled subregions indicate significant PNN density increase in the combined hemisphere analysis for that subregion in the Het (right) when compared to the WT (left) in the complex (A) and simple (B) paradigm. The darker the gray, the more significant the PNN density difference. A. Represents data from Figure 3.8. B. Represents data from Table 3.4.

APPENDIX

Behavior Protocols

All procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and approved by the University of Tennessee-Knoxville Institutional Animal Care and Use Committee.

Maternal Behavior Protocol (Chapter 2)

1. Behavior Set Up

- 1.1. Pair a female and male CBA/CaJ together in a large breeding cage.
- 1.2. Remove male from cage 1-2 weeks after pairing (or when female is visibly pregnant)
- 1.3. Based on level of pregnancy (judge on size and 21 days after pairing), place a WT and Het female (experimental surrogates) in the cage with the pregnant mother (between the ages of 10-12 weeks)
 - 1.3.1. Generally 1-5 days before birth of pups
- 1.4. The first day pups are born (P0) is the first day of the pup gathering paradigm.
 - 1.4.1. The paradigm is performed daily until P5 – after which the surrogates are perfused
- 1.5. Behavior is conducted in the home cage, which is placed inside a dark, sound-proof box and recorded using 30 fps video

2. Behavioral Paradigm

- 2.1. All adults are removed from home cage and placed in small holding cage for the duration of the behavior
 - 2.1.1. If there are more than 5 pups, the extra pups are also placed in holding cage with the adults
 - 2.1.2. There must be at least 3 pups to conduct behavior
- 2.2. Habituation Phase (5 minutes)
 - 2.2.1. All pups are placed in the nest and an adult is placed into the home cage
- 2.3. Isolation Phase (2 minutes)
 - 2.3.1. Pups are removed from the home cage

2.4. Retrieval Phase (10 minutes)

2.4.1. Pups are scattered around the cage in the 3 corners, then center of cage (if 4 pups), then home cage (if 5 pups)

2.4.1.1. Place initial pups in the locations farthest from the adult that is in the home cage

2.5. At the end of the retrieval phase, the adult is removed from the home cage and placed back in the holding cage

2.6. This step is repeated for all adults

2.7. Once all adults have been tested, they are all placed back in the home cage

Maternal Behavior Protocol (Chapter 3)

1. Behavior Set Up

1.1. Pair a female and male CBA/CaJ together in a large breeding cage if there are enough females from the litter for 6 WO behavior.

1.1.1. Note: you will not know the genotype of the females before the pairing as they will not be weaned yet – genotype at 3 weeks old

1.2. Remove male from cage 1-2 weeks after pairing (or when female is visibly pregnant)

1.3. Based on level of pregnancy (judge on size and 21 days after pairing), place a WT and Het female (experimental surrogates) in the cage with the pregnant mother (between the ages of 5-5.5 weeks so that they are ~6 weeks old at the time of P0)

1.3.1. If this timepoint is missed, discard the behavior – sometimes pregnancy is hard to time

1.3.2. Generally 1-5 days before birth of pups

1.4. The first day pups are born (P0) is the first day of the pup gathering paradigm.

1.4.1. The paradigm is performed on P0, P3 and P5 – after which the surrogates are perfused

1.5. Behavior is conducted in the home cage, which is placed inside a dark, sound-proof box and recorded using 30 fps video

2. Behavioral Paradigm

2.1. All adults are removed from home cage and placed in small holding cage for the duration of the behavior

2.1.1. If there are more than 5 pups, the extra pups are also placed in holding cage with the adults

2.1.2. There must be at least 3 pups to conduct behavior

2.2. Habituation Phase (5 minutes)

2.2.1. All pups are placed in the nest and an adult is placed into the home cage

2.3. Isolation Phase (2 minutes)

2.3.1. Pups are removed from the home cage

2.4. Retrieval Phase (5 minutes)

2.4.1. Pups are scattered around the cage in the 3 corners, then center of cage (if 4 pups), then home cage (if 5 pups)

2.4.1.1. Place initial pups in the locations farthest from the adult that is in the home cage

2.5. At the end of the retrieval phase, the adult is removed from the home cage and placed back in the holding cage

2.6. This step is repeated for all adults

2.7. Once all adults have been tested, they are all placed back in the home cage

Simple Tactile Tasks Behavior Protocol (Chapter 3)

1. Behavioral Set Up

1.1. Behavior box is placed in the sound-proof dark box (specifications of the box and inserts are in Chapter 3 description)

1.2. All behaviors except for adhesive are conducted in the dark, and are video recorded from the top and bottom view using 30 fps cameras

1.2.1. Adhesive is done in home cage, in the light, and recorded from the top view

1.3. Behavior starts at 5.5-6 weeks old and 11.5-12 weeks old

1.4. Perfusions are within 4 hours after completion of adhesive test

- 1.5. Alternate the order in which the animals are placed into the behaviors each day
2. Habituation (Day 1 & 2)
 - 2.1. Subject is placed in behavior chamber for 10 minutes
 - 2.2. Activity is recorded via video camera
 - 2.3. Subject is removed and box is disinfected with MB-10 to eliminate smells between animals
 - 2.4. Allow 5 minutes between each animal for the box to air out
3. Texture Discrimination (Day 3 & 4)
 - 3.1. Learning/Familiarization Phase (10 minutes)
 - 3.1.1. Place two identical textures (course) around the inserts then into the inner holes in the behavior chamber
 - 3.1.2. Begin to record then place subject in the behavior chamber
 - 3.1.3. Stop recording after 10 minutes (of the dark box door being closed)
 - 3.2. Retention Phase (5 minutes)
 - 3.2.1. Remove subject from chamber and place into the home cage
 - 3.2.2. Remove inserts and replace with a NEW course texture as well as novel texture (Day 3: medium grain ; Day 4: fine grain)
 - 3.3. Testing Phase (10 minutes)
 - 3.3.1. Start recording and indicate the location of the novel texture (hover hand over novel)
 - 3.3.2. Place subject in behavior chamber
 - 3.3.3. Stop recording after 10 minutes (of the dark box door being closed)
 - 3.3.4. Return subject to home cage
 - 3.3.5. Clean behavior chamber and textures
 - 3.3.5.1. Mist textures with MB-10 and allow to air dry
 - 3.3.5.2. Clean x 2 with MB-10 and allow to air dry for 5 minutes
4. Novel Object Recognition (Day 5)
 - 4.1. Learning/Familiarization Phase (10 minutes)

- 4.1.1. Place two identical shapes (cubes) into the outer holes in the behavior chamber
- 4.1.2. Begin to record then place subject in the behavior chamber
- 4.1.3. Stop recording after 10 minutes (of the dark box door being closed)
- 4.2. Retention Phase (5 minutes)
 - 4.2.1. Remove subject from chamber and place into the home cage
 - 4.2.2. Remove inserts and replace with a NEW cube as well as novel sphere
- 4.3. Testing Phase (10 minutes)
 - 4.3.1. Start recording and indicate the location of the novel object
 - 4.3.2. Place subject in behavior chamber
 - 4.3.3. Stop recording after 10 minutes (of the dark box door being closed)
 - 4.3.4. Return subject to home cage
 - 4.3.5. Clean behavior chamber and objects
 - 4.3.5.1. Mist objects with MB-10 and allow to air dry
 - 4.3.5.2. Clean x 2 with MB-10 and allow to air dry for 5 minutes
- 5. Adhesive Test (Day 6) *need two people*
 - 5.1. 3 trials at 3 minutes each, or until the adhesive is removed
 - 5.1.1. 3+ minutes is provided between each trial
 - 5.2. Home cage is placed in the behavior chamber (for easy recording)
 - 5.2.1. Remove bedding from home cage (as to not get stuck on the adhesive)
 - 5.2.2. Subjects are placed in clean new holding cages, individually, for easy access
 - 5.3. Subject is removed from holding cage and scruffed by experimenter 1
 - 5.3.1. Experimenter 2 presses record button before scruffing occurs, record all animals in one long video for each trial
 - 5.4. Experimenter 2 places adhesive between and slightly above the eyes of the subject
 - 5.5. Experimenter 1 places the mouse in the home cage, timer begins as soon as the experimenter releases the subject

- 5.6. Trial is over after the adhesive is removed or 3 minutes has past
- 5.7. Complete trial 1 with all subjects before starting the next trial
6. Perfuse subjects (Day 6)

Immunohistochemistry Protocols

Immunohistochemistry Antibodies

Primary/Secondary Antibody	Brand	Species	Dilution
(Primary) MECP2	Cell Signaling	Rabbit	1:1000
(Primary) Wisteria Floribunda	Sigma	Biotinylated	1:500
(Primary) Parvalbumin	Sigma	Mouse	1:1000
(Primary) GAD67	Cedar Lane	Rabbit	1:1000
(Secondary) Alexa 488	Invitrogen	Streptavidin	1:1000
(Secondary) Texas Red	Invitrogen	Rabbit	1:1000
(Secondary) Texas Red	Invitrogen	Mouse	1:1000
(Secondary) Cy5	Invitrogen	Mouse	1:1000
DAPI			1:1000

One Day Immunohistochemistry (Chapter 1)

Brains were sectioned in a sagittal fashion with a vibratome at room temperature in PBS the day before the IHC experiment. Sections were cut at 50 microns; every other section was used for the initial staining analysis. Remaining sections were stored at 4 degrees in 1X PBS solution.

1. Wash with 1X PBS, 3 X 5 min
2. Block with 10% normal goat serum (NGS) + 0.5% Triton-X in 1X PBS for 30 minutes
3. Incubate with antibodies (diluted in 5% NGS + 0.25% Triton in 1X PBS), 4 hours
4. Wash with 1X PBS, 3 X 5 min

Steps from here on are light-sensitive

5. Incubate with 2° antibodies (diluted in 5% NGS + 0.25% Triton in 1X PBS), 2 hours
6. Wash sections with 1X PBS for 2 X 5 min

7. Incubate with DAPI (1 uL of 5 mg/mL in 1 mL PBS) for 5 minutes
8. Wash sections with 1X PBS for 2 X 5 min, mount onto slide
9. Allow sections to air dry overnight before coverslip with Fluoromount-G

Two Day Immunohistochemistry (Chapters 2 & 3)

Brains were incubated for 12 hours in a 30% sucrose/PBS solution before sectioning on the freezing microtome. For Chapter 2, sections were cut in a sagittal orientation at 70-micron thickness and for Chapter 3 the brains were sectioned at 100 microns in a coronal orientation. Sectioning was done either the day before or day of the immunohistochemistry procedure.

Day 1:

1. Wash sections with 1X PBS, 3 X 5 min
2. Block with 10% normal goat serum + 0.5% Triton-X in 1X PBS, 3 hours
3. Incubate with antibodies (diluted in 1:1000 in 5% normal goat serum + 0.25% Triton in 1X PBS) overnight
4. Store remaining blocking solution at 4°C

Day 2: Light-sensitive

1. Wash sections in 1X PBS, 3 x 5 min
2. Incubate with 2° antibodies (diluted in 5% NGS + 0.25% Triton in 1X PBS), 4 hours
3. Wash sections with 1X PBS for 2 X 5 min
4. Incubate with DAPI solution for 5 min (1 uL of 5 mg/mL in 1 mL 1X PBS)
5. PBS wash 2 x 5 min 1X PBS wash
6. Mount tissues onto slide, and airdry overnight before coverslip with Fluoromount-G and seal with nail polish

PNN Quantification

High-Intensity PNN Imaging

All images for Chapters 2 & 3 were taken with a 10X objective, single plane on the Keyence epifluorescent microscope. Images were acquired at standard light sensitivity. Image settings for each experiment were based on the exposure from both the left and right hemisphere of the Het (specified in each experiment).

1. Establishing Exposure Setting

1.1. At 10X, the exposure setting that first created a saturated pixel in the PNN image was found, then the exposure setting that is one click down (on the software) was recorded as the ideal exposure setting for that hemisphere of that section.

1.2. This was done for each hemisphere of all SS1 containing sections for the brain.

1.3. Once all sections had been recorded, the most common LOWEST setting was used to image SS1 for that cohort.

1.3.1. Example: You have 7 1/25s, 30 1/20s, and 10 1/15s → You would image at 1/20

2. Each hemisphere is imaged separately (as it makes mapping easier)

2.1. Set 4 points using the XY-Stitching and Multipoint Tool

focus at each of these pointscan use more points if needed**

2.1.1. Point 1 is at the top of the brain close to the midline

2.1.2. Point 2 is at the beginning of the curve of the cortex (start of SS1 generally)

2.1.3. Point 3 is on the furthest (side) edge of the section, to make sure that all of the section is included in the image

2.1.4. Point 4 is at the bottom of the section, again to make sure all is included there

2.1.5. Repeat this step for all

3. Once these are completed, rename the files and stitch

4. Stitching the 10X images together using the BZ-X Analyzer

Mapping of Somatosensory Cortex (Sagittal)

Chapter 2 and IO Protocol ([dx.doi.org/10.17504/protocols.io.bcf8itrw](https://doi.org/10.17504/protocols.io.bcf8itrw)). ImageJ is required for this process. Available video tutorial: https://www.youtube.com/watch?v=7aNV_Kvipvk

1. Preparation

1.1. Download ImageJ, your SS1 images, and know the mm to pixel ratio for the SS1 images

1.2. Calibrate ImageJ to the scale based on the mm to pixel ratio for the image based on objective and magnification

1.2.1. Analyze → Set Scale → Measurement Values → check Global → exit image

2. Align map image to micrograph

2.1. Open image in ImageJ

2.2. Overlay map onto micrograph

2.2.1. Image → Overlay → Add Image → select map number at 20% opacity

2.2.2. Image → Overlay → To ROI Manager

2.3. Make the map fit the cortex

2.3.1. To rotate the fluorescent image to the map, deselect the map then go to Image → Rotate... and insert how many degrees you would like to rotate this image. Reselect the map and drag to fit again.

2.3.1.1. End goal: The curve of the striatum and the front edge of the hippocampus will line up. The front of the tissue should line up with the map as best as possible.

2.4. Flatten this overlay into one picture: Image → Overlay → Flatten.

2.4.1. To Save: File → Save As (tiff) → select the folder with the OG image → name this image differently

3. Outline Whole SS1

- 3.1. Increase image brightness so you can see the border of the striatum and hippocampus
 - 3.1.1. Image → adjust → brightness/contrast (slide the contrast tool)
- 3.2. Select the polygon tool and outline the whole somatosensory cortex.
- 3.3. Save outline to ROI Manager (press 't')
- 3.4. Rename the ROI: In ROI manager
 - 3.4.1. select ROI and then the 'Rename' function. Name SS1 Area.
- 3.5. Acquire Area Measurement using the Measure button
- 3.6. To Save in the same folder as flattened image
 - 3.6.1. In ROI Manager → More → Save
4. Outline SS1 Subregions
 - 4.1. Using the same tools as 3, outline each individual subregion
 - 4.2. Save to ROI Manager (press 't')
 - 4.2.1. Rename the outline with the corresponding subregion
 - 4.3. Save each subregion to the folder with the flattened image individually
 - 4.4. Record subregion area (same as 3.5)

Mapping of Somatosensory Cortex (Coronal)

For Chapter 3, we decided to section coronally, so our criteria for mapping the images were different from sagittal. We also separated the cortex into 'top' and 'bottom' layers and high-intensity PNNs were quantified in these different layer designations as well as subregions.

1. Preparation/Calibration
 - 1.1. Open micrograph in ImageJ
 - 1.2. Calibrate ImageJ to the mm to pixel ratio for your image
 - 1.2.1. Analyze → Set Scale → Insert Specified Values for your image
 - 1.3. Make sure 'global' is checked! Click 'OK'
 - 1.4. Close out the image
2. Align map to micrograph

- 2.1. Drag the image and corresponding map number into ImageJ/FIJI
- 2.2. Click on image
- 2.3. Overlay map onto this image
 - 2.3.1. Image → Overlay → Add Image → select map image from drop box → change to 20 in opacity
- 2.4. Before clicking anything else check to see that the map overlay is now in the ROI Manager
- 2.5. Stretch or shrink map to fit map onto image
- 2.6. Open Brightness/Contrast window
 - 2.6.1. Image → Adjust → Brightness/Contrast
- 2.7. Adjust image brightness and contrast or click 'auto' to see hippocampus and/or CPu clearly

Before the next step make sure to DESELECT the map overlay
- 2.8. Rotate image to better align with the CPu and/or Hippocampus
 - 2.8.1. Image → Transform → Rotate
 - 2.8.2. Keep track of # of degrees each time you make a rotation
 - 2.8.3. Sum up the rotation values once you have map perfectly, this will be needed for PNN quantification later
- 2.9. Flatten the Image once the overlay is perfectly aligned with the micrograph
 - 2.9.1. Image → Overlay → Flatten; Save this new file as a TIFF – this will be used to outline the subregions
- 2.10. For best fit of map onto image:
 - 2.10.1. Align top, bottom, midline and edge of tissue as much as possible
 - 2.10.2. Align CPu and Hippocampus (if applicable), for lower map #s alignment relies heavily on the CPu
3. Outline SS1 Subregions and layers
 - 3.1. Open the corresponding flattened image for the micrograph
 - 3.2. Outline one subregion at a time using the polygon tool
 - 3.2.1. Top Layer

- 3.2.1.1. Outline from the Pia (top of cortex) which is the top boundary
- 3.2.1.2. The bottom boundary should hug the bottom part of the dense PNN layer (layer 4)
- 3.2.1.3. Save this in the ROI manager and rename as 'Region TL'
- 3.2.2. Bottom Layer
 - 3.2.2.1. For the top boundary, use the TL bottom boundary
 - 3.2.2.2. For the bottom boundary, the line should overlay with either the corpus collosum or the caudate putamen
- 3.3. Obtain the area measurement for each ROI
 - 3.3.1. ROI Manager → Measure → Results

Quantification of High-Intensity Perineuronal Nets

1. Image Preparation
 - 1.1. Open micrograph in ImageJ
 - 1.2. Rotate micrograph to specified rotation
 - 1.2.1. Image → Transform → Rotate... → Insert Specified Value
 - 1.3. Open Brightness/Contrast Panel (Image → Adjust)
 - 1.4. Drag ROI's for the subregion outlines for which PNNs will be quantified
2. Quantification Tools
 - 2.1. Increase the contrast slider to maximum (B&C Panel)
 - 2.1.1. Make sure to 'reset' the contrast every couple times you slide it
 - 2.2. Select the 'point' tool
 - 2.2.1. Make sure the tool displays ONE crosshair, not multiple. If it shows multiple, right-click to switch
 - 2.2.2. When selecting a PNN, click on the PNN then press 't', this will save that point into your ROI Manager
3. Counting PNNs
 - 3.1. All PNNs that are present at maximum contrast should be quantified
 - 3.1.1. These will have the typical donut or circle-like shape

- 3.1.2. To qualify, at least 80% of the original PNN structure must be present at high contrast (see 80% instructions)
- 3.2. Count PNNs separately for each subregion and layer specification.
- 3.3. To save counts:
 - 3.3.1. Deselect any ROI in the ROI Manager
 - 3.3.2. Save the file to the original folder as “Region XL PNNs”
 - 3.3.2.1. This SHOULD save as a ZIP folder, make sure that it is not a blank folder or PDF
 - 3.3.3. Attain number of PNNs by clicking Measure in the ROI Manager – this will also give you the area of the subregion
 - 3.3.4. Close the Measure window and delete the ROIs, you can then move onto the next region
- 3.4. 80% Rule
 - 3.4.1. Using the segmented line tool, trace the PNN at high contrast, close that segment
 - 3.4.2. Reset the contrast on the image, using the segment tool complete the outline of the PNN that can be seen at the base setting
 - 3.4.3. In ROI Manager, click on Measure to acquire the length of both segments
 - 3.4.4. Divide the high contrast segment by the total length measurement to determine if 80% of the PNN is present at high contrast

VITA

Dana Elizabeth Layo-Carris was born in Boston to Terry and Kimberly Layo and grew up in Sandwich, a small town on Cape Cod in Massachusetts. She graduated from Sandwich High School before moving to Salem, Virginia to attend Roanoke College in 2012. She participated in the Undergraduate Research Assistantship Program from 2012-2016, and the summer research scholar program in 2014, under Dr. W. Gary Hollis in the Chemistry department synthesizing self-healing polymers. She also acted as a teaching assistant in many chemistry courses throughout her time at RC. She was a visiting research scientist at the Woods Hole Oceanographic Institute during the summer of 2015 under Dr. Daniel Repeta. At WHOI she helped identify the structure of a high molecular weight phosphorus containing molecule which, when consumed by microbes in the Pacific, contributes significantly to atmospheric carbon dioxide levels. After graduating in 2016 with a Bachelor of Science in Biochemistry, Dana elected to continue her education at the University of Tennessee at Knoxville with the intent to pursue more biology-focused research. There, she joined the lab of Dr. Keerthi Krishnan, studying the neural mechanisms underlying Rett Syndrome pathology. During her tenure, Dana has presented her work at multiple conferences (both in-person and virtual) and participated in the BCMB graduate student organization as graduate student recruiter and acting president during the COVID-19 pandemic. She has also participated in community outreach through the GSO as well as Skype a Scientist and connecting with local elementary schools. Despite enduring extended animal facility closures, laboratory building moves and the coronavirus pandemic, she still managed to complete this dissertation. Dana has accepted a post-doctoral position with Dr. Elizabeth Bhoj at Children's Hospital of Philadelphia and the University of Pennsylvania Perelman School of Medicine, continuing her work on pediatric genetic disorders.