

Sensory-evoked activity in somatosensory cortex as a model to probe cortical plasticity in a
mouse model of Rett syndrome

by

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BSc, Azad University of Tehran, 2003
MSc, Azad University of Tehran, 2007

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Supervisory Committee

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Abstract

Rett syndrome (RTT), a severe neurodevelopmental disorder, affects females resulting from loss-of-function mutations in the X-linked transcription factor methyl-CpG-binding protein 2 (MECP2). RTT patients show severe verbal, motor, respiratory, and intellectual impairments. We studied two forms of activity-dependent plasticity in *Mecp2* mutant mice to better understand the loss of MECP2 function in neuronal circuit and sensory processing.

Sensory deprivation was applied by trimming one whisker to 3 mm to study long-term cortical plasticity in *Mecp2*^{-y} mice. Intrinsic optical signaling (IOS) imaging showed the neuronal response to wiggling a non-trimmed was consistent from day 0 to 14 but reduced for the trimmed whisker by $49.0 \pm 4.3\%$ in wild type (WT) and $22.7 \pm 4.6\%$ ($p=0.0135$) in RTT mice.

Primary hindlimb (HL) somatosensory cortical responses to vibratory stimulation were assessed by IOS and intracortical local field potential (LFP). Responses were assessed before, during and, after 1 hour of repeated HL vibratory stimulation (100Hz, 1sec, ISI 6 sec) in symptomatic male (4-6 week), female (10-12 month) and pre-symptomatic young female (4 week) RTT model mice. After 1-hour, cortical responses to test vibrations were reduced by approximately 40% in RTT and WT mice as assessed by both methods. Recovery of the IOS responses (1 sec vibration at 100Hz) and LFP (300 μ m below pia, 7 stimuli, 100msec ISI) were tested at 15 min intervals for 1 hour after ceasing the repeated stimulation. Reduced responses persisted for at least 60 min in WT but recovered to 90-100% of normal within 15-30 min in RTT. Analysis of the LFP responses within the test train indicated that the reduced cortical sensitivity during and after continuous stimulation resulted primarily from an increase in adaptation during the 7-stimulus test train rather than a reduction in the response to a single vibratory stimulus in all groups.

Retention of this increased STA is the primary cause of the persistently reduced tactile response in young WT female mice, while in RTT mice the rapid recovery of tactile sensitivity was due to the return of STA to lower, baseline levels. Male RTT mice exhibited a marked increased excitability to the first stimulus in the test train resulting in hypersensitivity to a single vibration by 45 minutes. Old females exhibited the same pattern of adaptation and recovery but retention of adaptation was less pronounced in both WT and RTT compared to younger animals suggesting an age-dependent reduction in neural plasticity may mask deficits specific to RTT. Recording sciatic nerve sensory afferent activity did not reveal any STA, persistent adaptation or sensitization of peripheral afferent endings in any groups.

I propose persistent sensory adaptation mediated by increased short-term adaptation may reflect enhanced feedback by inhibitory elements of circuits within the sensory pathway. The rapid recovery of responsiveness in young female RTT mice may therefore reflect a deficit in the capacity for activity dependent plasticity to consolidate and thus could provide a platform to understand the causes of learning and cognitive deficits in RTT patients.

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List of Abbreviations

BOL: Blood oxygen saturation level	P: Peak response
CNS: Central nervous system	PA: Persistent adaptation
EEG: Electroencephalogram	PNS: Peripheral nervous system
ERP: Event related potential	PV: Parvalbumin-expressing neurons
E/I: Excitation/Inhibition	RTT: Rett syndrome (in the introduction section)
GAD1: Glutamate decarboxylase 1	RTT: Male Rett model mice (in the result section)
GABA: Gamma-aminobutyric acid	RTT-F: Symptomatic female Rett model mice
HL: hindlimb	RTT-YF: Pre-symptomatic female Rett model mice
IOS: Intrinsic Optical Imaging	S1: primary somatosensory cortex
ISI: Inter stimuli intervals	SER: Sensory Evoked Response
L: Cortical layer	SNAPs: Compound Sensory Nerve Action Potentials
LTP: Long term potentiation	SOM: Somatostatin-expressing neurons
LTD: Long term depression	STA: Short-term adaptation
LFP: Local Field Potential	TRD: Transcription Repression Domain
MBD: Methyl-CpG-binding domain	TRN: Thalamic Reticular Nucleus
MECP2: Methyl-CpG binding protein 2	V1: Primary visual cortex
<i>Mecp2</i> ^{-y} : Hemizygous male Rett mice	VB: Ventrobasal complex
<i>Mecp2</i> ^{+/-} : Heterozygous female Rett mice	WT: Wild type male mice
N: Negative peak response	WT-F: 10-12-month-old wild-type female mice
NMDA: N-methyl-D-aspartate	

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Chapter 1: Introduction

1.1 Rett Syndrome

Rett syndrome (RTT; OMIM identifier #312750) is a neurodevelopmental disorder resulting from loss-of-function mutations in the X-linked gene (Xq28), methyl-CpG-binding protein 2 (*MECP2*) (Amir et al., 1999). RTT was described initially in the 1960s by an Austrian pediatrician, Dr. Andreas Rett, and widely recognized later in the 1980s by Dr. Bengt Hagberg (Hagberg, 1983). RTT is a severe autism-associated neurodevelopmental disorder affecting 1 in 10,000 live female births (Laurvick et al., 2006). Female patients develop normally until 6 to 18 months, after which they start showing symptoms such as loss of speech, purposeful hand movement, acquired microcephaly, seizure, and autistic features. In females, random X chromosome inactivation results in somatic mosaics with normal and mutant *MECP2*. Males born with mutant *MECP2* are very rare, and they show neurological deficits from birth. Male patients show severe congenital encephalopathies and mostly die within two years (Schüle et al., 2008). However, 99.5% of all cases are sporadic. Due to *de novo* mutation in the *MECP2* gene, rare familial cases of RTT due to X-chromosomal inheritance from a carrier mother have been seen. Studies on female patients with different mutations in *MECP2* showed that the origin of the mutation is parental, which explains the high female: male ratio in the patients (Trappe et al., 2001). The severity of RTT can vary widely based on the type of mutation of *MECP2*.

1.2 *MECP2* mutations

There are 500 mutations linked to RTT (Miltenberger-Miltenyi & Laccone, 2003), mainly on exon three and four (Four exons in total (Reichwald et al., 2000)). These mutations are primarily single-point mutations in the coding sequence. Eight of these mutations are more common in RTT patients, and they are located in the region of the protein called a “hot spot” (Figure. 1). Most of these common mutations occur at arginine (R) residue. The majority of these mutations target the methylation function of MECP2. However, there are two mutations in RTT patients which do not affect this function (T158M and R168X), indicating MECP2 functions other than methyl- CpG binding are critical for neurodevelopment (Adkins & Georgel, 2011).

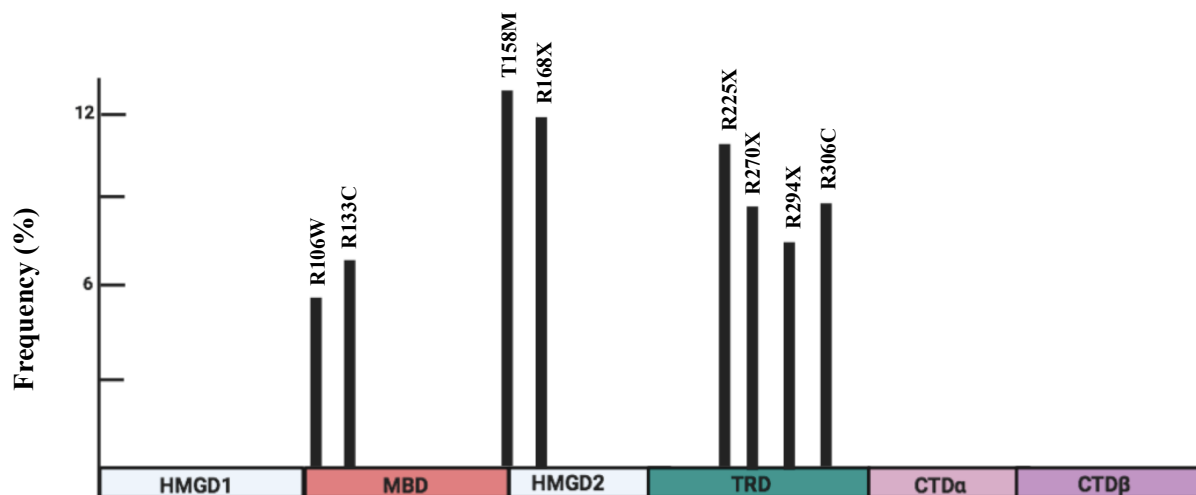


Figure 1, Location and frequency of *MECP2* mutation in Rett syndrome patients (hot spots).

Most of the point mutations are located in the gene's TRD (Transcription Repression Domain) region. R= Arginine, W= tryptophan, C= cysteine, T= threonine, M= methionine, X= stop codon. (numbers show the location of the mutation. For example, “R106W” means point mutation at residue 106, which arginine replaced by tryptophan (modified from Adkins, 2011).

1.3 Clinical feature and stages in Rett patients

RTT is recognized as having a stage of normal early development followed by subsequent regression (Figure. 2). However, an early developmental delay in RTT females has been reported (Einspieler et al., 2005; Gillentine et al., 2017).

Most of the patients are short, underweight, and microcephalic. Head growth slows down two to four months after birth, with the main symptoms developing after 12 to 18 months (Neul, 2012). Symptoms can include hypotonia, jerkiness in limb movement (Dolce et al., 2013), and limited social interaction (lack of interest in others). However, some of these symptoms vanished, and individuals become more social with age, except for those diagnosed with an autism spectrum disorder (Ip et al., 2018). RTT patients show verbal, motor, and intellectual impairments. This regression often results in significant motor disability, with some teenage female patients becoming dependent on a wheelchair for the rest of life (Dolce et al., 2013).

Seizures are one of the common symptoms of RTT; 48% of RTT patients have episodes. Seizures have an age-related onset and are infrequent before the age of two (Glaze et al., 2010).

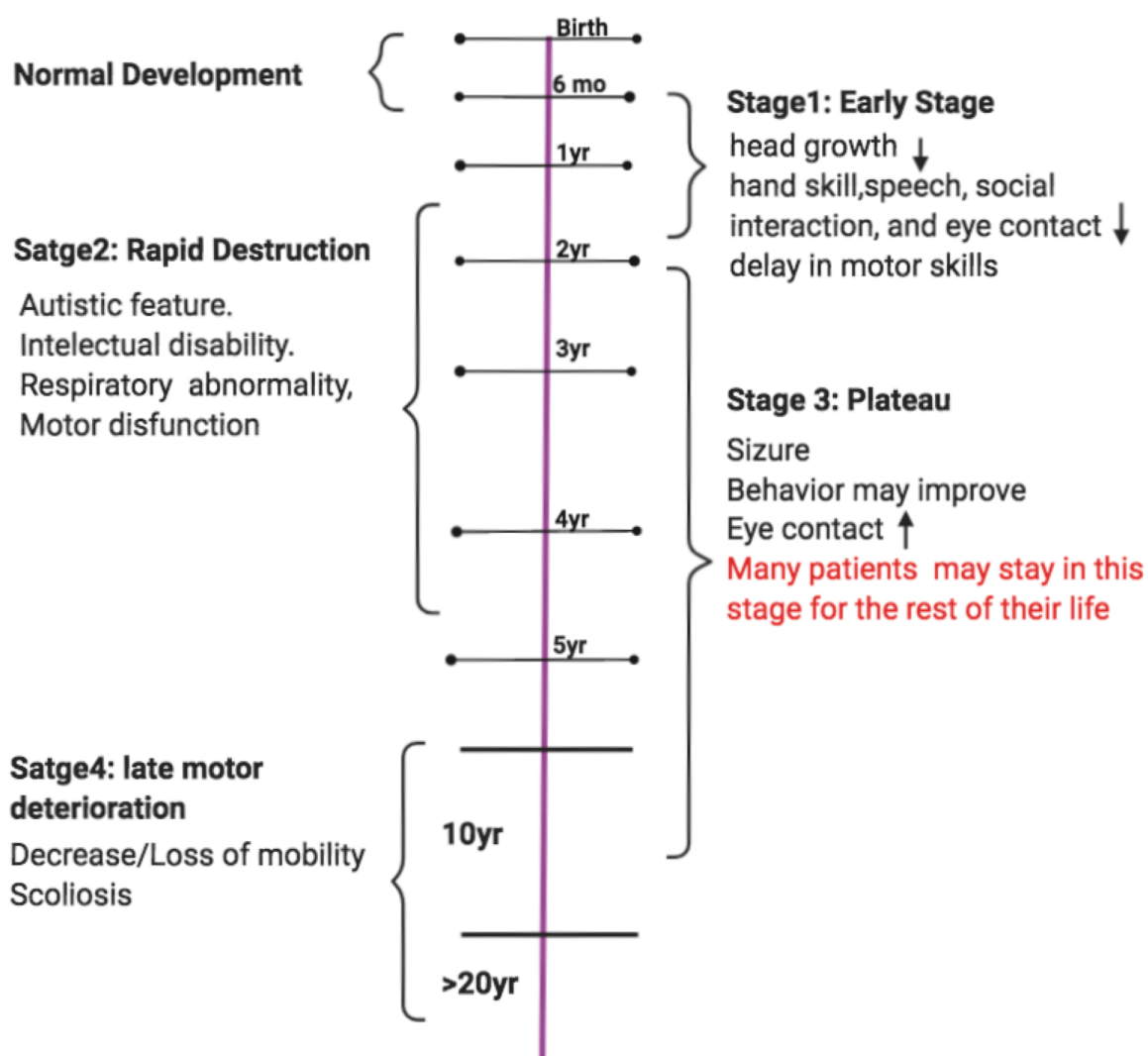


Figure 2, Different stages in Rett syndrome development

Patients with X-linked gene methyl-CpG-binding protein 2 (MECP2) mutation were born normal. They start showing symptoms after 6 to 18 months. Rett syndrome is a progressive syndrome and includes four stages. In stage one, which begins at 6-month-old, head growth, hand skill, speech, social interaction, and eye contact is reduced. The delay in motor skills is noticeable in this stage too. At stage two, which starts at 1.5- years old, the autistic feature appears, more dramatic symptoms, including breathing and motor dysfunctions, emerge. Some patients go to stage three, and they stay in that stage for the rest of their life. In this stage, patients show seizures, but some behavior, like eye contact, is improved. The symptom could get worse by moving to stage four. In this stage, patients lose their mobility and have to use a wheelchair.

1.4 Sensory impairment in RTT

In many neurological disorders like autism spectrum disorder, schizophrenia, and RTT, dysfunction of sensory information has been reported (Goffin et al., 2014).

1.4.1 Electroencephalogram (EEG) is altered due to *MECP2* mutation

EEG is abnormal at some time during the stages of RTT (Kálmánchey, 1990). For example, in stage one, EEG is usually normal, particularly during sleep. In stage two, the background activity slows even more, and there are some sharp-wave discharges during non-REM sleep and wakefulness. These sharp waves are usually followed by stereotypic hand movement. At stage three, the background activity slows down more, and sharp-wave discharges still occur in wakefulness. At the end of stage three, a generalized slow spike-wave pattern is seen in wakefulness (Kálmánchey, 1990; Glaze, 2005).

Mutation of *Mecp2* in mice leads to abnormality in EEG recordings similar to what has been seen in patients. Male *Mecp2*-null mice and female heterozygous mice show reduced frequencies in theta rhythm and spontaneous abnormal rhythmic discharges (Goffin et al., 2012; D'Cruz et al., 2010).

1.4.2 Somatosensory system impairment in RTT

There is some evidence regarding abnormality in response to pain (Downs et al., 2010) and touch (Bhattacharjee et al., 2017) in RTT patients.

A study in 2009 showed abnormal responses to pain. In 75.2% (total of 646 RTT patients) of patients, the response was abnormal, with 65% showing a decreased sensitivity to pain. This group of patients had a different type of *MECP2* mutation from the rest, meaning the pain sensitivity may be related to the type of mutation (Downs et al., 2010).

Somatosensory evoked potential (SEP)¹ is altered in RTT patients. A “giant”- SEP is observed in patients younger than nine years old and not in older patients (Yoshikawa et al., 1991; Sysoeva et al., 2020). Myoclonus (jerk contraction of a group of muscles) is seen in some RTT patients. In these patients, motor evoked potentials showed typical latency while the somatosensory evoked potentials were enlarged. C-reflex was hyper-excitabile and prolonged, meaning RTT patients have prolonged intercortical delay of the long loop reflex (Guerrini R, 1998).

At the early stage of RTT, the abnormality is seen only at the cortical level, while by aging, the brainstem and spinal cord become involved (Glaze, 2005). A longer latency of the N1 peak has been reported in RTT patients (Nomura et al., 1987). Measuring the SEP in 11 females between the ages of three and 18 showed that the cortical N1 latency is significantly delayed in females older than five years. In females more than nine years old, recordings from the brainstem and cervical cord indicated delays in P14 components of the brainstem and N13 components of the cervical cord (Glaze, 2005).

Studies on RTT animals reported enhancing inhibition in specific layers of the somatosensory cortex (Dani et al., 2005; Lo et al., 2016), which could be the reason for the abnormal somatosensory cortex function (Sysoeva et al., 2020).

1.4.3 Visual System Impairment in RTT

There are different studies regarding the abnormality of visual evoked potentials (VEPs) in RTT patients. Although VEP abnormality was not observed in 18 months to 4.5 years old children (Kálmánchey, 1990), an increase in latency of primary positive response (P1) in RTT girls was seen (Bader et al., 1989). VEP in children between the ages of 22

¹ SEP: electrical potential occurs in the cortex after stimulation of a sensory organ and measurable by surface electrodes

months and nine years old showed N1 and P1 latencies, and no change in N2 amplitude did not change in RTT (LeBlanc et al., 2015). This finding indicates that the visual pathway remains intact with information gets to the cortex normally. However, P1 amplitude was decreased significantly in RTT patients compared to an age-matched control group. In addition, due to the increase of latency of the N2 peak, 50% of RTT patients showed multiple positive peaks (LeBlanc et al., 2015).

VEP abnormality has been reported in Rett model mice. The older females who have more severe symptoms showed smaller P1 amplitude (LeBlanc et al., 2015) and increased the N1 amplitude (Liao et al., 2012).

1.4.4 Auditory System Impairment in RTT

RTT patients can experience disruptions in their verbal communication abilities, and this has led to an interest in uncovering the potential existence of auditory processing impairments in RTT. Studies on subcortical auditory neuronal responses indicated no evidence for peripheral hearing loss in RTT patients or animal models (Sysoeva et al., 2020; Adcock et al., 2020).

Cortical auditory event-related potential (ERP)² in RTT patients is impaired. Prolonged latency in ERP in early and late responses indicates deficits at the thalamocortical level (Bader et al., 1989). However, impairment in auditory processing is observed at a higher level due to an increase in ERP amplitude in RTT females between the ages of 4 and 21 (Sysoeva et al., 2019).

Altered ERP has also been observed in Rett model mice. A reduction of amplitude but an increase of N1 and P2 peak latency during auditory stimulation was seen only in

² ERP= measuring the brain response in response to a specific sensory, motor or cognitive event.

symptomatic mice, showing the disruption of sensory processing happens in an age-dependent manner (Goffin et al., 2012). Since the short-latency auditory brainstem responses (ABR) are not abnormal, the initial audition remains intact, and the impairment site is central (Liao et al., 2012).

1.4.5 MECP2 (methyl-CpG binding protein 2) functions in the brain

The *MECP2* gene is expressed in all tissues, but reaches near histone levels of abundance in neurons ($\sim 16 \times 10^6$ molecules per nucleus) (Skene et al., 2010). In addition to the loss of function mutations in *MECP2* that cause RTT, duplication of *MECP2* also causes a distinct neuronal developmental disorder (Neul et al., 2010). This shows the nervous system is susceptible to MECP2 doses and any changes in the level of MECP2 protein can lead to neurological and psychiatric problems.

MECP2 protein is a global transcriptional factor (Nan et al., 1998; Fuks et al., 2003; Yasui et al., 2007) and RNA splicing regulator (Young et al., 2005). In the brain, level of MECP2 is high during the embryonic phase, which then increases steadily during a period of intense synaptic formation and maturation in the first few days after birth. Levels eventually plateau in humans at ten years (Shahbazian et al., 2002) and mice in five weeks (Skene et al., 2010). The MECP2 level remains high during adulthood which shows the role of this protein in mature brains (Figure.3).

The increase of MECP2 expression follows a distinct spatial pattern. Cajal-Retzius cells began to express MECP2 first, followed by the deeper cortical layers and finally more superficial layers (Shahbazian et al., 2002).

The reduction of brain weight in RTT patients is associated with abnormally small neurons (reduced dendritic branching, reduced spine density, and defects in spine morphology)

packed densely (Armstrong, 2005). The decrease in brain volume is not generalized. The cerebral hemispheres are affected more than the cerebellum. There is a selective reduction in the volume of female RTT patients' parietal and temporal lobes while the occipital cortex is preserved (Carter et al., 2008).

Smaller brain size is observed in symptomatic mice model, with the overall thickness of the cortex in *Mecp2*^{-y} mice being reduced at eight weeks of age. There is a substantial reduction of the cortex thickness in layers 2/3, and pyramidal neurons in this layer are smaller with less complex dendritic arborization than wild-type animals (Kishi & Macklis, 2004). In heterozygous female Rett (*Mecp2*^{+/-}) mice, *Mecp2*⁻ neurons in layer 5 (L5) have smaller soma and shorter basal dendritic length than *Mecp2*⁺ neurons (Rietveld et al., 2015). The development of neuronal circuits relies on the growth of dendrites and spines. Several studies showed impairment in neuronal connections and synaptic transmissions due to disruption of *MECP2*, which indicates that MECP2 is essential in modulating synaptic functions.

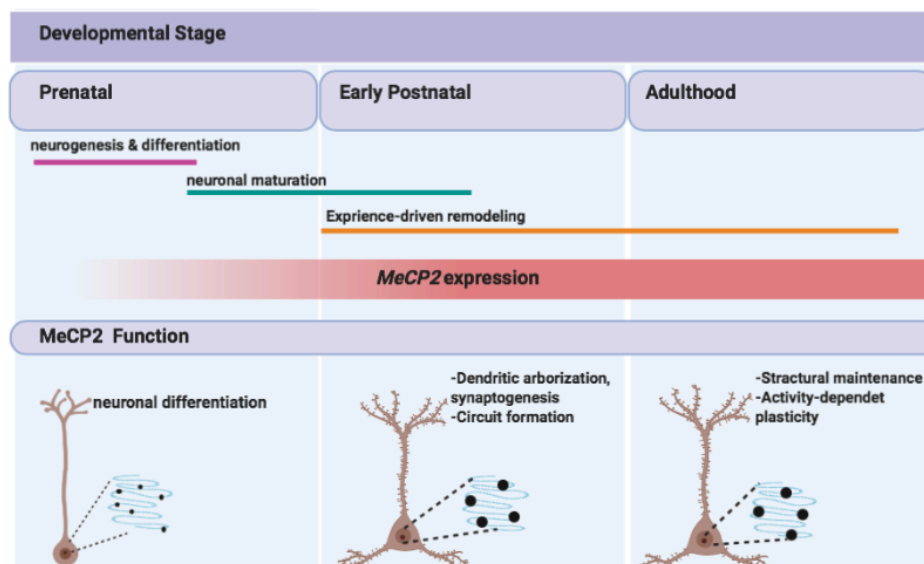


Figure 3, MECP2 rules during neuronal development.

In the early embryonic stage, MECP2 regulates neuronal differentiation and, after birth, neuronal maturation. MECP2 initiates the chromatin clustering (black dots in the figure, mature neurons contain fewer and denser chromocenters); therefore, MECP2 participates in chromatin structure establishment in mature neurons. In adulthood, it is a critical factor in the maintenance of neuronal function. MECP2 keeps the chromatin structure and regulates the transcriptomic profile in neurons. Furthermore, MECP2 keeps a permissive state for stimulus-dependent gene transcription and regulates cognitive function. (modified from (Karaca et al., 2019))

1.5 Neuronal circuit alteration as a result of *MECP2* loss of function

Individual RTT patients and animal models show region-specific alterations in excitatory/inhibitory (E/I) balance. A reduction in spontaneous activity of L5 pyramidal cells in cortical slices of null male mice results in shifting the cortical E/I toward inhibition in this area which is due to a decline in the number of the excitatory synapses to these cells and not change in the intrinsic firing rate of neurons (Dani et al., 2005). There is an E/I imbalance in L5 and L6 of the prefrontal cortex in *Mecp2*-null male and heterozygous female Rett model mice due to a decrease in the spontaneous excitatory tone and an increase in the inhibitory input onto pyramidal neurons (Asgarihafshejani et al., 2019). Reduced excitation in some areas of the cortex may lead to motor and cognitive impairments. CA3 of the hippocampus in symptomatic *Mecp2* knockout male mice described impaired E/I in favor of excitation over inhibition in this area, which may contribute to seizures in the RTT human and mouse models (Calfa et al., 2015).

The mechanism underlying this regional specificity is not precise, but there are two possibilities. The first possibility is that *MECP2* may have different functions in different parts of the brain. The second possibility is that some neurons and circuits may be more sensitive to mutations of the *MECP2* gene. *MECP2* interacts with methylated DNA. The landscape of DNA methylation varies in different cell types and between excitatory and inhibitory neurons (Mo et al., 2015). Therefore, *MECP2* may exert different molecular actions in various cell types, resulting in specific transcriptional changes with *MECP2* mutation. *Mecp2* mutation in forebrain excitatory neurons causes a milder phenotype in mice than animals with a lack of *Mecp2* in inhibitory neurons (Meng et al., 2016; Gemelli et al., 2006). Re-expression of *Mecp2* in the inhibitory neurons and not in excitatory neurons is sufficient to rescue the motor coordination deficit in Rett mice (Meng et al., 2016; Ure et al., 2016).

Although only 20% of the total neurons in the brain are GABAergic neurons (Figure.4), it has been suggested that they contribute to 80% of the workload. Mutation of *Mecp2* in different GABAergic neuronal subtypes causes different and non-overlapping phenotypes. For instance, mice with a lack of MECP2 in somatostatin-expressing neurons (SOM⁺) neurons show seizures, while mice with a lack of MECP2 in parvalbumin-expressing neurons (PV⁺) neurons exhibit sensory, motor, and memory deficits (Ito-Ishida et al., 2015).

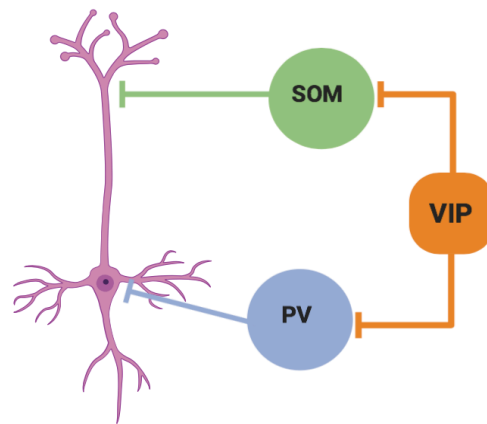


Figure 4, Schematic connection of interneurons subtypes to pyramidal cells in the cortex.

There are three types of interneurons in the cortex that influence pyramidal cells directly. Somatostatin-expressing interneurons (SOM⁺) and parvalbumin-expressing interneurons (PV⁺) inhibit the pyramidal neurons by targeting the dendritic and soma of pyramidal neurons, respectively. Vasoactive intestinal polypeptide-expressing interneuron (VIP⁺) disinhibits the pyramidal neurons by inhibiting SOM⁺ and PV⁺ neurons.

MECP2 has a high impact on PV⁺ neurons. The number of PV⁺ puncta and PV⁺ neuron connections is increased in the visual cortex of *Mecp2*^{-/-} mice compared to wild-type animals (Krishnan et al., 2015; Durand et al., 2012). Additionally, the level of NMDAR subunit expression is changed in these mutant mice, as they show a higher NR2A: NR2B ratio in PV⁺ neurons. This imbalance of the subunit ratio suggests abnormally accelerated NMDAR maturation which causes hyper-connectivity of these cells (Mierau et al., 2016).

Mutation of *MECP2* also affects the level of the KCC2:NKCC1 ratio. Two neuronal cation-chloride cotransporters, KCC2 (export K⁺/Cl⁻) and NKCC1 (import Na⁺/K⁺/Cl⁻), set

the transmembrane chloride gradient and determine the maturation of GABAergic inhibition (Kaila et al., 2014; Blaesse et al., 2009). The levels of KCC2 relative to NKCC1 are reduced in the cerebrospinal fluid samples from RTT (Wilson et al., 2012). The expression of KCC2 is reduced in V1 of null *Mecp2* male mice, which causes the reversal potential of GABA to be less negative compare to wild-type control animals. This effect is reversible by treating with IGF1 (Insulin-Like Growth Factor1) (Banerjee et al., 2016). In addition, GABA is less hyperpolarizing in iPSC-derived neurons collected from RTT patients (Tang et al., 2016).

Overall, mutation of *MECP2* reduces the GABA synthesis level, Glutamate decarboxylase 1 (GAD1) and GAD2, miniature postsynaptic current, and reduction on the KCC2:NKCC1 ratio which all cause the reduction of inhibition onto pyramidal cells. It seems, due to the homeostatic response in neuronal circuits (Nelson & Valakh, 2015), the PV⁺ neurons make hyper-connection to pyramidal cells to compensate for the reduction of inhibition onto pyramidal cells. These all together cause a joint decrease of excitation and inhibition in the cortical circuit of RTT (Ip et al., 2018).

Maintaining the E/I balance in brain regions is crucial for every aspect of brain function. It helps quick responses to even tiny changes in synaptic inputs due to sensory stimulation, and altered E/I balance impacts cortical plasticity in neural circuits.

1.6 MECP2 and neuronal plasticity

For many years, it was believed that the brain does not make significant changes after a certain point in time. However, it has been proved that the brain is capable of changing and developing throughout a lifetime. The brain is plastic, and the term “neuronal plasticity” is used to describe the ability of the brain to change the connectivity during development, learning, or injury and potentially healing itself. Many studies indicated that changes in experiences (Ko et al., 2013) and learning (Chen et al., 2015) cause the neurons in the

microcircuit to modify their functional connectivity and their responses (Turrigiano & Nelson, 2004). Overall, the development of inhibitory and excitatory circuits and retaining the E/I balance require activity-dependent mechanisms (Nelson & Valakh, 2015). These mechanisms are known as synaptic plasticity, divided into short-term and long-term plasticity based on their time scale.

Short-term plasticity, including synaptic depression and synaptic facilitation, lasts for milliseconds to minutes (Regehr & Abbott, 2004).

Long-term plasticity includes Hebbian synaptic (LTP/LTD³) plasticity, which involves changes that last from hours to days, weeks, and even months (Abraham, 2003). Donald Hebb proposed Hebbian plasticity in the 1940s; coincident presynaptic and postsynaptic activity (positive feedback) will result in the gain of a synapse and results in storage of the information in the brain (learning).

Homeostatic plasticity takes the brain to the original state (Turrigiano, 2011). Homeostatic plasticity sets a negative feedback-driven mechanism that sustains synaptic strength in neurons and circuits at appropriate levels (Fox & Stryker, 2017). Homeostatic plasticity stabilizes the firing rate of neurons to regulate synaptic strength through synaptic scaling, which means quantal amplitude (the amplitude of postsynaptic response to a single vesicle of neurotransmitter) of all synapses onto a postsynaptic neuron can scale up or down in response to long-lasting changes in neuronal activity (Turrigiano & Nelson, 2004). Therefore, an increase in neuronal activity causes a decrease in quantal amplitude, and a reduction in activity results in an increase in amplitude. Taken together, these types of plasticity cause the brain to have dynamic responses to experience.

³ LTP: long term potentiation, LTD: long term depression

There is some evidence of impairment of Hebbian plasticity due to *Mecp2* mutation at hippocampal Schaffer collateral-CA1 synapses (Asaka et al., 2006), and at the motor and sensory origin of the neocortex in symptomatic male Rett mice (Moretti et al., 2006; Dani & Nelson, 2010).

MECP2 has a critical role in the homeostatic plasticity of scaling up in neocortical neurons in response to a reduction in firing or sensory drive (Blackman et al., 2012). Altered experience-dependent refinement of circuits resulting from dysfunctional hemostatic machinery may be a core functional mechanism underlying autism and RTT (Elbert & Greenberg, 2013; Nelson & Valakh, 2015).

Interneurons have a crucial impact on cortical plasticity. Conditional deletion of *Mecp2* from PV⁺ neurons of the visual cortex (V1) in mice causes the failure of ocular dominance plasticity during the critical period, while selective mutation of *Mecp2* in either pyramidal cells or SOM⁺ neurons did not have the same effect. This shows the role of PV⁺ cells in cortical plasticity during the critical period. Mutation of *Mecp2* conditionally from PV⁺ cells reduced the excitatory transmission from pyramidal cells to PV⁺ cells and, as a result, decreased the inhibition in the cortex. Restoring inhibition via infusion of GABA agonist diazepam restores plasticity (He et al., 2014). Treatment with IGF1 abolish impairment in experience-dependent plasticity in the visual cortex of Rett model mice (Castro et al., 2014) and stabilized the underlying circuit, possibly through effects on the homeostatic mechanism (Blackman et al., 2012) or the inhibitory system in the cortex (Castro et al., 2014).

Overall, how neurons wire together in a brain and make the brain function is dependent on the proper functioning of excitatory and inhibitory neurons and maintenance of E/I balance, all of which require MECP2. In this project, simple approaches were designed to increase our current knowledge regarding the role of MECP2 on the neuronal circuit. Since RTT is a top

candidate to investigate abnormal neuronal plasticity due to E/I imbalance, it was decided to study cortical plasticity under natural brain conditions by applying long-term and short-term plasticity protocols *in vivo* in Rett model mice.

1.7 Mouse Models of Rett Syndrome

Non-vertebrates (including fruit flies and worms) do not have the MECP2 gene, while all vertebrates have the gene (Hendrich & Tweedie, 2003). This makes mouse models essential for gaining a better understanding of RTT impairments. Several different types of Rett model mice have been designed (Ribeiro & MacDonald, 2020). Some models contain common point mutations in RTT patients. Two models have a total lack of MECP2 protein; *Mecp2^{tm1.1Bird}* (Guy et al., 2001) and *Mecp2^{tm1.1Jae}* (Chen et al., 2001).

Mecp2^{tm1.1Jae}, the model used in the current project, has a deletion of exon 3 of the *Mecp2* gene in all cells. As RTT affects females predominantly, female model mice (*Mecp2^{+/-}*) are the most clinically suitable model to study RTT syndrome. It is essential to consider that due to X chromosome inactivation, the lack of MECP2 protein might differ between individual animals, increasing the phenotype variation. However, the hemizygous mice models (*Mecp2^{-/-}*) show a consistent phenotype due to the complete lack of MECP2 protein. Consequently, *Mecp2^{-/-}* might be a better model to investigate the underlying mechanism of the lack of MECP2 in patients. Therefore, this project was done on both male and female Rett model mice.

Mecp2^{-/-} mice are phenotypically normal until four weeks old. The symptoms start around four weeks old, including irregular breathing patterns, seizure, hindlimb clasping, small brain volume, and low body weight. They die around eight weeks old, meaning there is a short window to perform experiments on this group.

Heterozygous *Mecp2*^{+/-} mice live an average lifespan and usually do not show the symptoms until four to six months old due to the mosaic pattern of mutant and nonmutant cells resulting from X-chromosome inactivation (Robinson & Pozzo-Miller, 2019). Since the female model is phenotypically normal until six months old, it was decided to study both pre-symptomatic (2-month-old) and symptomatic (12-months-old) female Rett model mice in this project, which provides us with more insight into the impact of disease progression on the cortical plasticity impairments.

1.8 Project objectives

Although several studies have shown abnormalities in RTT brains in both humans and mice, it has not been clear how the lack of MECP2 affects neural circuit performance during behavior.

To investigate the effects of loss of MECP2 function in neuronal circuit function and sensory processing, we studied two forms of activity dependent plasticity in somatosensory cortex in male and female Rett model mice lacking functional MECP2 (*Mecp2*^{tm1.1Jae}), using Intrinsic Optical Imaging (IOS) and Local Field Potential (LFP) recording.

We applied a sensory deprivation paradigm by trimming whisker C1 on the right-side snout for 14 days to assess how chronically reduced input to a cortical region affects its responsiveness to subsequent sensory input. We examined the effects of repeated activation of cortical inputs producing sensory adaptation by continuous stimulation of the hindlimb (HL) for one hour, and recovery from adaptation following stimulus cessation.

Finally, to investigate whether the location of observed plasticity (sensory adaptation) in the sensory pathway (from HL to the somatosensory cortex) occurs somewhere within the central nervous system (CNS) or is expressed partially or perhaps fully at the level of the

peripheral afferent endings we recorded vibration evoked afferent volleys from the sciatic nerve.

2 Long-Term Plasticity Following Sensory Deprivation is Reduced in Male Rett Model Mice

2.1 Introduction

2.1.1 Overview

We evaluated long-term cortical plasticity due to sensory deprivation in 4-6-week old male Rett model mice and age-matched wild-type mice. Long-term cortical plasticity was induced by trimming whisker C1 for 14 days. The evoked cortical response was recorded with Intrinsic Optical Signaling (IOS) imaging from the corresponding barrel cortex.

2.1.2 The brain has a topographic map

Neurons are not organized randomly in the cortex. In some areas of the brain (somatosensory, auditory, and visual cortex), neurons are arranged topographically based on their responses to specific stimuli of a particular sensory structure. The location of a neuron in a region of the brain predicts the type of information that a particular neuron processes the best.

The spatial distribution of sensory receptors in the somatosensory cortex forms a somatotopic map that corresponds to the overall shape of the body from feet to head to hands. The size of the corresponding area of the somatosensory cortex is not proportional to the body size. For example, areas of the somatosensory cortex that respond to stimuli of hands and lips are more significant than torso and proximal limbs. This arises from the fact that manipulation of facial expression and speech requires fine motor control and high-resolution sensory information. Therefore, the larger portion of both central and peripheral circuits is essential to processing the vital information from those locations (Figure.5).

2.1.3 Whisker to barrel cortex signaling

In rodents, whiskers are moved back and forth at high frequency (10-20 Hz) (Petersen, 2019) to scan the environment (whisking). Since they are nocturnal animals and often live in tunnels, the tactile sensory information provided by whisking enables them to gather essential details about the spacing and texture of surrounding objects (Petersen, 2007). The tactile signals from each whisker evoke neuronal activity in a discrete column in the barrel cortex.

The whisker arrangement on both sides of the snout (mystacial pad) is highly stereotyped between mice. Whiskers are located in 5 rows (A-E) and several arcs or columns (7 to 9). There are four additional whiskers, called α , β , γ , and δ , that are arranged between the rows, caudal to the mystacial pad (Brecht et al., 1997). In mice, each mystacial whisker is individually represented in the somatosensory cortex by an obvious large-scale (~ 200 - $300\ \mu\text{m}$) structure called the barrel. The layout of the barrel in the cortex is almost identical to the design of whiskers on the snout (Woolsey & Van der Loos, 1970). For example, whisker C2 is the second whisker in the C row (Figure.6A) represented by barrel C2 (Figure. 6B).

Follicles, the structure that anchor whiskers to the skin, are innervated by the peripheral branches (~ 200 cells) of the trigeminal ganglion (Dörfl, 1985). Deflection of a whisker opens mechano-gated ion channels in nerve endings causing an action potential to fire in the primary sensory neurons, which project centrally via the infraorbital branch of the trigeminal nerve. These sensory neurons make excitatory glutamatergic synapses in the trigeminal nucleus of the brainstem. Barrelettes, somatotopic structures of trigeminothalamic neurons in the trigeminal nucleus, receive substantial input from a single whisker (Veinante & Deschênes, 1999). The trigeminal neurons project to the ventral posterior medial nucleus (VPM) of the contralateral thalamus. The excitatory VPM neurons cluster in discrete somatotopic barreloids (Simons & Carvell, 1989). Axons from individual VPM barreloids project mainly but not exclusively to

layer 4 (L4) of the somatosensory cortex to form the barrel map. Individual neurons in the VPM mainly project to just one cortical barrel. For example, deflection of whisker C2 causes a neuronal response largely restricted to the C2 barrel cortex (Figure. 6C).

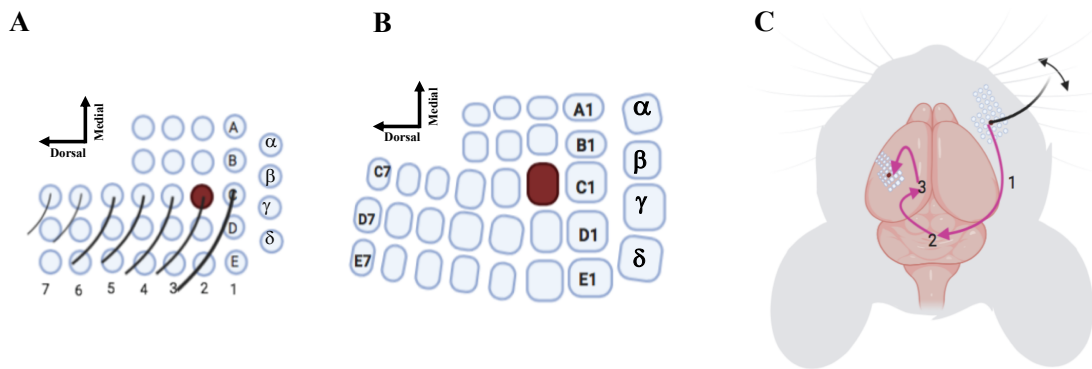


Figure 6, Whisker to barrel cortex pathway

A) Whiskers on a whisker pad are located in 5 rows (A-E) and 7 to 9 columns on the snout. In addition, there are 4 whiskers called α , β , γ , and δ which are located between rows. **B)** The pattern of the barrel is almost identical to the layout of whiskers on the snout of rodents. **C)** An action potential fires in the primary sensory neurons of the infraorbital branch (1) of the trigeminal nerve due to deflection of a whisker which then projects to the trigeminal nucleus of brainstem (2). Sensory input from brainstem transfers to ventral posterior medial nucleus (VPM) of the contralateral thalamus (3). Axons from VPM project mainly to layer 4 of somatosensory cortex and form the barrel map (modified from (Petersen, 2019)).

2.1.4 Barrel cortex circuit

The soma of spiny stellate cells in L4 of the barrel cortex are located mainly at the barrel borders. This arrangement results in low cell density at the barrel hallows, which causes the barrel cortex to be readily visible with transmitted illumination (Woolsey et al., 1975). Each of the barrels are separated by thin septa in mice (Woolsey et al., 1975). A column in the barrel cortex is defined as a thickness of the cortex (1200 μm), laterally bound by the dimension of the barrel (200-300 μm). Each barrel contains approximately 6,500 neurons (about 85% are excitatory glutamatergic neurons and 15% are inhibitory GABAergic neurons), connected in an individual barrel and connected to surrounding barrel columns and other brain regions (Petersen, 2019).

In rodents, sensory signals from whiskers reach the somatosensory cortex through lemniscal and paralemniscal pathways (Figure. 7A). Each pathway originates from a distinct thalamic nucleus and targets various barrel cortical areas (Feldmeyer, 2012).

The primary sensory input from VPM arrives in L4 of the barrel cortex. L4 neurons only have local axonal arborizations. Therefore, they primarily send excitatory signals to other cortical layers of the barrel column. In an individual barrel column, layer 2/3 (L2/3) is innervated by a dense column of axons from L4 excitatory neurons. L2/3 contains excitatory pyramidal neurons with dendrites expanded to L1 and extensive local axonal arborization in L2/3 and layer 5 (L5). L2/3 axons spread horizontally across the barrel cortex (Narayanan et al., 2015) (Figure. 7B).

The corticothalamic neurons in layer 6 (L6) project to the thalamus (VPM and posterior medial nucleus (POm)). L6 neurons also innervate L5 (Kim et al., 2014) and they project broadly across the somatosensory cortex. It appears that L6 neurons serve a modulatory rather than driving function (Petersen, 2019).

The GABAergic inhibitory neurons in L2/3, L4, and L5 also receive thalamic excitatory input. The inhibitory neurons that are parvalbumin-expressing (PV⁺) provide feed-forward, feedback, and lateral inhibition in local circuits of the cortex. The presence of these inhibitory neurons cause the wiring of excitatory neurons to be strong and specific to a particular stimulus (Sermet et al., 2019).

During whisker deflection, the firing rate of neurons in VPM and POm is increased. The excitatory sensory inputs from the thalamus arrive at L4 and L5/6 first (Crochet et al., 2011) which subsequently causes the activation of L2/3 excitatory and inhibitory (PV⁺) neurons. The excitatory neuronal response from S1 travels to other brain regions, including the

primary whisker-related motor cortex (Petersen, 2019). L5 and L6 send feedback signals to VPM and POm (Staiger & Petersen, 2021).

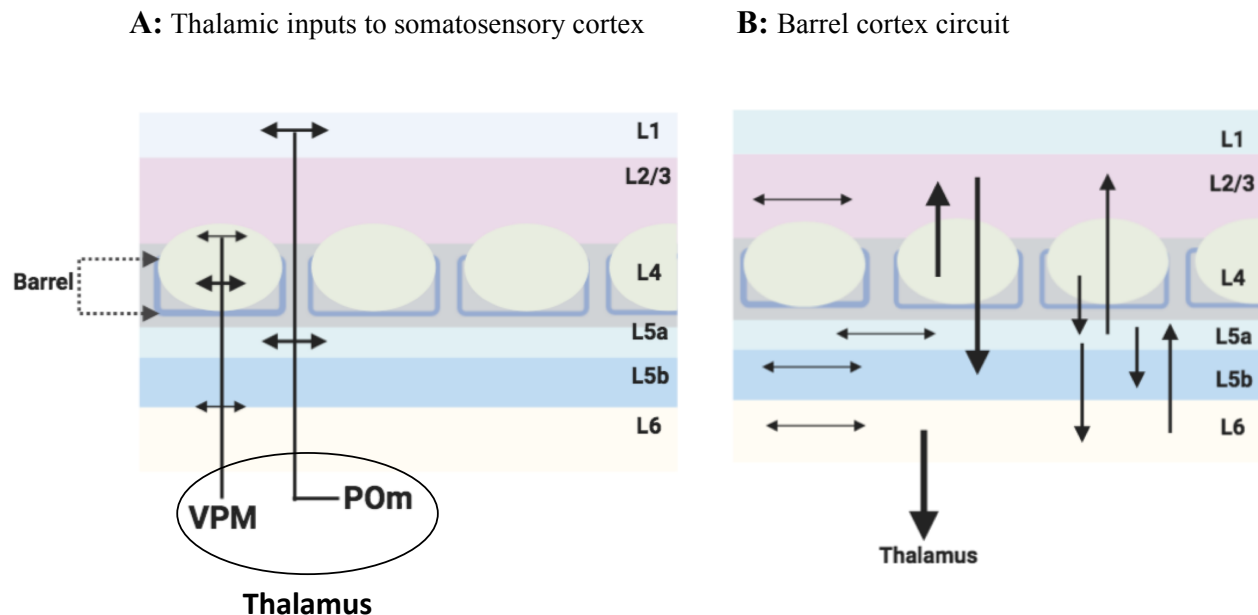


Figure 7, Schematic picture of sensory input signaling to the barrel cortex and within cortex layers

A) Thalamus sends the sensory input, from deflection of a whisker, to the barrel cortex via 2 pathways (lemniscal and paralemniscal). Lemniscal pathway: ventral posteromedial nucleus (VPM) receives inputs from principle trigeminal nucleus (PrV) of the brainstem and forwards them mostly to layer 4 (L4) in the hollow of barrel cortex and some to the border of layer 5 (L5b) and layer 6 (L6). Paralemniscal pathway: sensory inputs from spinal trigeminal nucleus (SpV) of brainstem transfer to the posterior medial nucleus of the thalamus (POm) and project to layer 1(L1) and upper layer 5 (L5A) but not to the barrel related L4. **B)** Sensory inputs transfer from L4 mainly to layer2/3 (L2/3) and also L5 and L6. The sensory inputs also transfer horizontally in L2/3, L5 and L6. L5 and L6 send feed-back signals to the thalamus.

2.1.5 Sensory deprivation dependent plasticity in rodent somatosensory cortex

We know a brain can change as a result of an environmental change or experience. A sensory experience can drive plasticity in sensory maps and cause cortical remapping. In a classic example of map plasticity, when a subset of input is deprived, representation of the deprived input is contracted within the map, and spared inputs are expanded (Polley et al., 1999). The primary somatosensory cortex (S1), specifically the barrel cortex of rodents, is a classic model system to investigate sensory processing and plasticity in mammals.

In the early stages of development, the anatomical structure of the barrel cortex of rodents is fixed. Still, the neuronal responses can be changed in an experience-dependent manner, even in adulthood. For example, trimming a particular whisker causes a contraction of the corresponding barrel and expanding the surrounding barrels. The cortical representation of surrounding whiskers (intact whiskers) expands into the region of the cortex that is no longer activated by the trimmed whisker (Polley et al., 1999). This type of plasticity is reversible; re-growing the whisker will revert the spread of cortical activity.

Following whisker trimming, the sensory inputs to L4 of the barrel cortex are reduced. As a result, the excitatory inputs to L2/3 decrease, causing sensory depression in L2/3 neurons (Bender et al., 2006). L2/3 neurons above the barrel do not get sufficient input from the L4 underlying barrel; instead, they receive substantial input from neighboring barrels (through inter-column neuronal connections). As a result of Hebbian type plasticity, the neuronal connections from neighboring barrels to L2/3 pyramidal neurons of a deprived whisker get stronger. After a few days (4-7 days), the corresponding cortex of the deprived and intact neighboring whiskers shrink and expand, respectively (Feldman & Brecht, 2005). The experiment in the current chapter was designed based on this well-established model.

Cortical plasticity due to whisker trimming is similar to monocular visual deprivation, where the remaining open eye “takes over” the cortical territory which is normally occupied by the deprived eye (Petersen, 2007) (However, monocular visual deprivation does not remain plastic in developed brains). Excitatory synapses from L4 to L2/3 and horizontal L2/3 to L2/3 cross-columnar synapses are the main sites of plasticity. It appears that LTP and LTD are the primary synaptic plasticity mechanisms occurring at the site of expansion of the surrounding and shrinkage of trimmed whiskers in the S1 receptive field (Foeller & Feldman, 2004) (Figure.8).

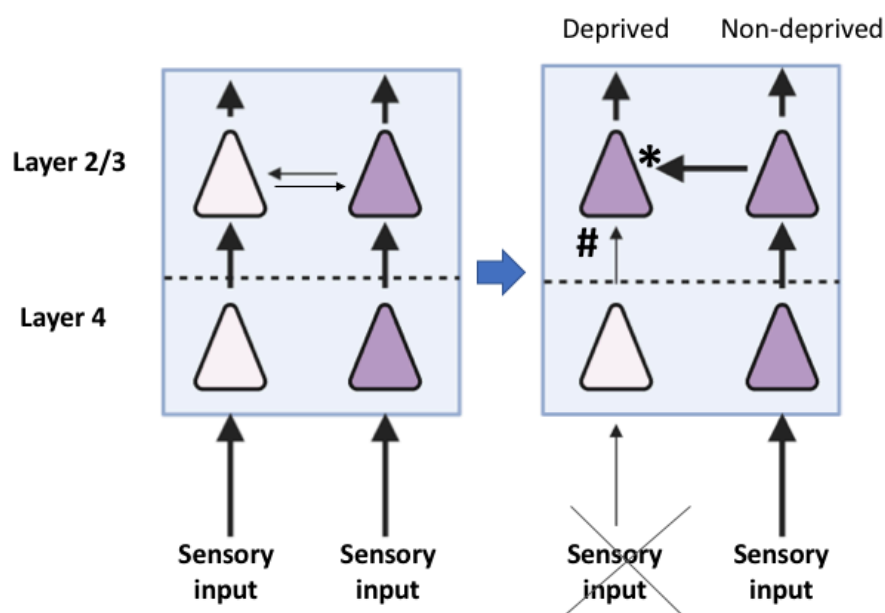


Figure 8, Synaptic plasticity as a result of sensory input deprivation

As a result of sensory deprivation, sensory input reduces to the corresponding cortical barrel. This causes Long-term Depression (LTD) at the synaptic site of layer 4 to layer 2/3 of the corresponding barrel (#). Horizontal connections between layer 2/3 pyramidal neurons (*) get stronger (Long-term Potentiation) after sensory deprivation. Therefore, the remaining neighboring whiskers take over the cortical territory, which is normally occupied by a deprived whisker.

2.1.6 Rationale

The consequences of MECP2 dysfunction for learning and memory have been shown in several studies (Na et al., 2013; Hao et al., 2016; Li et al., 2017; Kee et al., 2018; Karaca et al., 2018; Robinson & Pozzo-Miller, 2019). Spatial memory is examined using various maze tests (Moretti et al., 2006; Kee et al., 2018). Each maze is designed to examine how well an animal can remember the location of food or rewards. Since male *Mecp2* null (*Mecp2*^{-/-}) mice have developed motor dysfunctions (including difficulties in swimming), it is difficult to assess the cognitive performance through routine memory experiments. To avoid interference of motor disfunction with learning, memory, and cognition impairment in the Rett mice model, we conducted a more straightforward task (cortical plasticity) in *Mecp2*^{-/-} mice. This allowed us to investigate the consequences of *Mecp2* loss-of-function for neural circuit performance on the barrel cortex in 4 to 6-week-old male Rett model mice and age-matched wild-type mice as a result of a whisker deprivation. The primary somatosensory cortex, specifically the barrel cortex of rodents, is a classic model system to investigate sensory processing and plasticity in mammals (Bender et al., 2006; Foeller & Feldman, 2004; Sermet et al., 2019; Drew & Feldman, 2009; Martin-Cortecero & Nuñez, 2014).

There were several reasons we choose to study the barrel cortex. First, the whisker to barrel signaling pathway is well known. In addition, the one-to-one correspondence between facial whiskers and the array-like anatomical barrel column in the primary somatosensory cortex (S1) enabled us to induce and study map plasticity by trimming an individual whisker. Furthermore, S1 is located superficially, allowing live optical imaging of neuronal activity in response to a whisker trimming.

The barrel response to the deprived whisker declines which is reversible after the whisker grows back (Polley et al., 1999; Feldman & Brecht, 2005). The general result of

sensory deprivation on S1 through whisker trimming is usually a reduction of neuronal activity in the corresponding receptive field of the barrel cortex to the trimmed whisker and an increase in the neuronal activity of the surrounding barrels (adjacent one). We hypothesized that the cortical sensory map (barrel cortex) plasticity in Rett model mice would be reduced compared to the plasticity in wild-type mice. We expected that there would be either a decreased reduction in neuronal activity in the Rett model mice compared to the wild-type mice, or that more time would be required in the Rett model mice for the same amount of reduction in neuronal activity as the wild-type mice. To test this hypothesis, *Mecp2* knockout (*Mecp2^{-/-}*) mice model were used. *Mecp2^{-/-}* mice undergo a period of normal development that is followed by severe progressive neurological dysfunction and eventually death around eight weeks of age. The hemizygous mice model (*Mecp2^{-/-}*) has a complete lack of MECP2 in all cells compared with heterozygous female model mice (*Mecp2^{+/-}*), which show 50% expression of wild-type *Mecp2*. Therefore, due to the complete absence of MECP2 protein in the male model (*Mecp2^{-/-}*), we expected to see the more pronounced impairment of cortical plasticity, if any, in this group compared to female *Mecp2^{+/-}* mice.

To study the level of cortical plasticity in the barrel cortex of male Rett mice, whisker C1 was trimmed, and whisker B2 was used as an untrimmed control whisker in each animal. The cortical activity was recorded by Intrinsic Optical Signaling (IOS) imaging. IOS imaging generates *in vivo* images of cortical activity, which can be assessed through the thinned, intact skull with spatial resolution sufficient to map single barrels. This method has been used to study the plasticity of a single whisker's functional representation in developing and adult rodents (Knutsen et al., 2016).

2.2 Material and methods

2.2.1 Animals

Mice with non-functional *Mecp2* gene were bred from heterozygous females (*Mecp2*^{+/-}) with wild-type (C57BL/6) males. The heterozygous Rett females (*Mecp2*^{+/-}) were obtained from UC Davis MMRRC (*Mecp2*^{tm1.1Jae}). They have a lack of exon three on the *Mecp2* gene. The Hemizygous males (*Mecp2*^{-y}) are born normal but start showing symptoms around weeks 4-6. They usually die around eight weeks of age. 4-6-week old male animals were used for this set of experiments. The control group included the age-matched wild-type males (C57BL/6). All mice were housed in 12h/12h dark/light cycles. 18 *Mecp2*^{-y} and 14 wild-type mice were used for this set of experiments. PCR was performed to confirm the genotype of the experimented animals.

2.2.2 Surgery

Each animal was anesthetized with 1.5% isoflurane: air mixture. The depth of anesthesia was monitored by the animal's response to a toe pinch. During the surgery, the body temperature was measured with a rectal probe and maintained between 36.5°C to 37°C by a thermostatically controlled heating pad. A circular region of the skull over the barrel cortex (right hemisphere) was cleaned, and a thin layer of clear dental cement (C&B -Metabond) was applied over the exposed (intact) skull with a 3-mm diameter coverslip placed on the cement. Once the dental cement dried, surface vessels were clearly visible through the intact skull. The cranial window was attached to the skull three days prior to imaging sessions.

The benefits of “intact skull imaging” are: 1) Surgery is not invasive, and recovery time after the surgery is short. 2) Keeping the skull intact minimizes heartbeat-induced and breathing-related brain movement and therefore reduces the noise.

2.2.3 Whisker stimulation

The principal whisker (C1) on the left side of the snout was trimmed to 3-mm in length and inserted into a short tube glued onto pencil lead attached to a piezoelectric. The whisker was stimulated at 20Hz for 1 second (The stimulation frequency used in the experiments is selected from the range of frequencies close to the one that mice use to explore the environment) and displaced ~0.5 mm in both rostral and caudal directions. Cortical activity corresponding to the whisker stimulation was recorded for 3 seconds.

Whisker C1 was trimmed to 3 mm and cut every other day to keep the whisker's length the same during the experiment period (14 days). Whisker B2 was considered an untrimmed control whisker. It was also inserted into a plastic tube, glued to a piezoelectric, the end of which was positioned at the same distance ~1 mm from the snout. Rett syndrome is a neurodevelopmental disorder, and the experimental male Rett mice were at the stage of developing the symptoms. Whisker B2, an untrimmed whisker, was used as a control to ensure that any changes observed in the activity of C1 barrel cortex from trimming whisker C1 could not be explained by changes due to either Rett symptom development or cranial window quality changes over the length of experiment. Furthermore, whisker B2 is not adjacent to C1; therefore, change in the activity of the C1 barrel cortex does not affect the activity of neurons at the B2 barrel cortex. The whiskers were stimulated on day 0 (before trimming), 7, 10, and 14 days after first trimming. Cortical activity was imaged on the right primary somatosensory (barrel) cortex (Figure. 9).

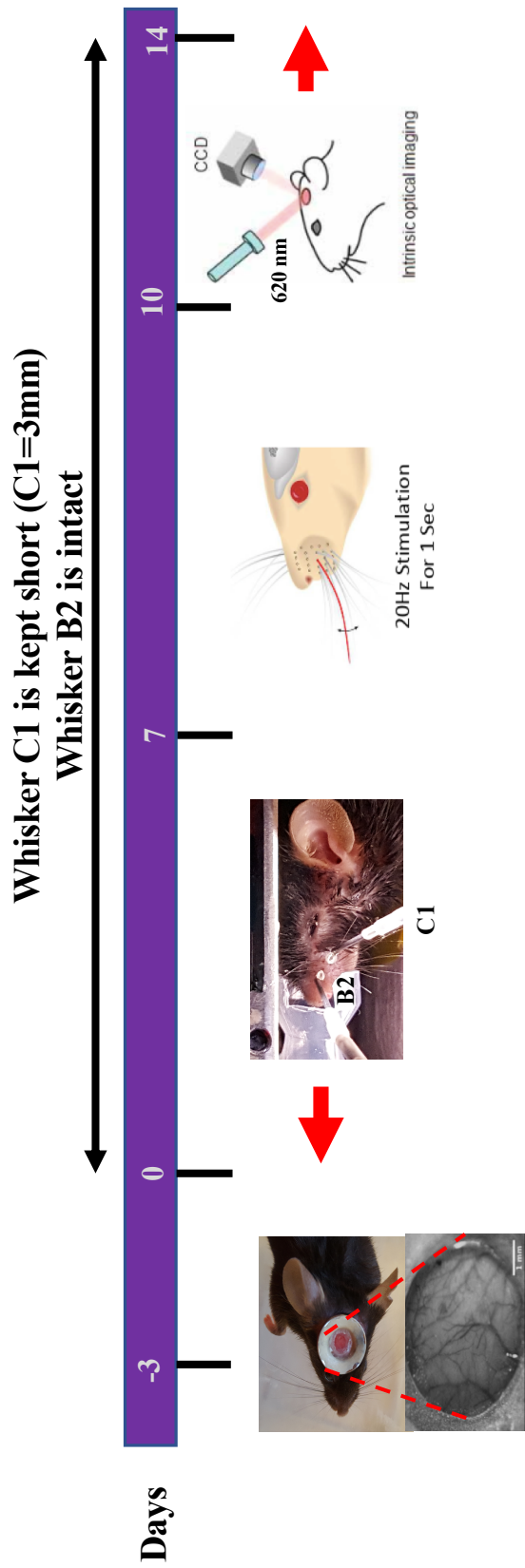


Figure 9, Long-Term plasticity inducing protocol (sensory deprivation)

Cranial window (intact skull) is implanted on right hemisphere above barrel cortex 3 days before imaging. At day 0 (before trimming) both C1(trimmed) and B2 (control) whiskers on left side of the snout are wigged separately with a piezo-based device (20Hz for 1 sec, 0.5mm rostral/caudal). Change in reflectance of 620 nm light is monitored and analyzed. Whisker C1 is trimmed to 3 mm (re-trimmed every 2 days), and IOS resulting from whisker stimulation is imaged again at day 7, 10 and 14.

2.2.4 Intrinsic Optical Signaling (IOS) imaging

During the imaging sessions, animals were anesthetized under isoflurane (1% isoflurane for wild-type animals and 0.5% for mutant animals): air mixed. Body temperature was monitored and maintained at $36.5 \pm 0.5^\circ \text{C}$ using a rectal probe and a thermostatically controlled heating pad. The heart rate, breathing rate, and level of blood oxygen saturation of animals were monitored with an oximeter during the imaging sessions. Prior to IOS imaging, an image from the cerebral vessel, illuminated by green light ($\approx 550\text{nm}$), was obtained with a 2X objective lens (NA 0.14). Red light ($\approx 620\text{nm}$) was used to record the activity of the corresponding barrel cortex to contralateral whisker stimulation. The imaging plane was focused about 300-350 μm below the cortical surface to minimize the contribution from surface blood vessels (Frostig & Chen-Bee, 2009). Whiskers were stimulated with 20Hz for one second and displaced 0.5mm in both rostral and caudal directions. One second of spontaneous activity was collected prior to stimulation onset, one second of evoked activity during stimulation, and one second of activity after the stimulus. In total, the brain activity was recorded for three seconds in each trial, and trials were repeated 20 times with a 14-second inter-trial interval (Figure.10).

2.2.5 Data analysis

Each trial contains 300 image frames (3 seconds of cortical activity) (Figure. 10). The reflection prior to stimulation onset (R_{baseline}) was obtained by averaging the first 100 frames (Figure.11B). A stack of 300 frames was divided over the R_{baseline} image. Finally, frames 150 to 300 were averaged as the final image ($R_{\text{post response}}$) (Figure.11C). The evoked cortical response starts to appear 0.5 sec after stimulation onset; therefore, frames 150 to 300 were averaged to visualize the final image.

Two times median filter with radius 100 was applied on the baseline image (Z-stack average of frames 1 to 100) and subtracted from the reflection ratio value ($R_{\text{post response}}$). The final image was smoothed with a Gaussian filter ($\delta=2$). The neuronal activity in the corresponding barrel cortex was thresholded to 80% of the maximum signal amplitude for a particular area. Then the average pixel value in the limited area was measured, which is correlated to the activity of neurons in that area (Figure. 11E). This value, referred to as the fractional change in reflectance (ΔR) throughout this dissertation, was plotted.

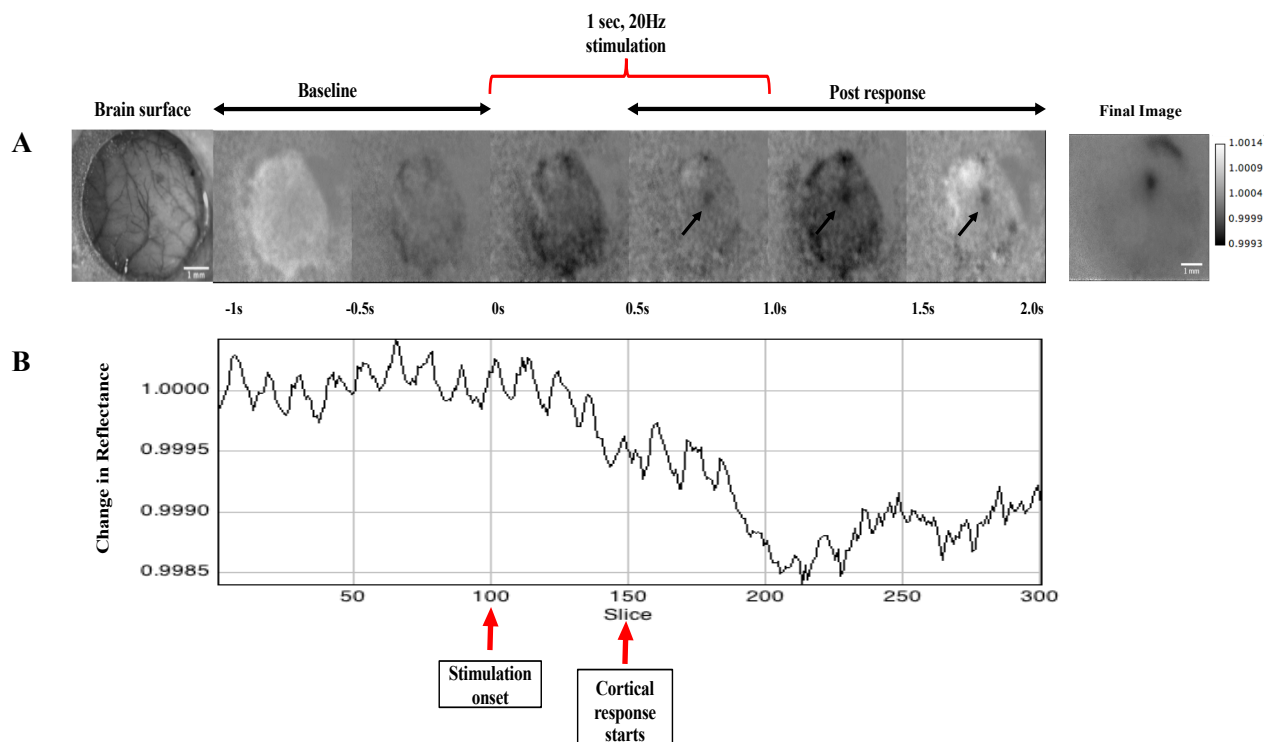


Figure 10, Recording the cortical activity for 3 seconds in each trial

A) Cortical activity was recorded for 3 sec, 1 sec before the onset of stimulation (Baseline), 1 sec during the stimulation (wiggling the whisker), and 1 sec after the end of stimulation. Cortical response (black dot/ black arrows) to the sensory stimulation starts 0.5sec after stimulation onset (post response). B) 300 frames (3sec) were captured. Stimulation of the whisker began at frame #100, and the response began to appear at frame #150. To measure the evoked brain response to the sensory stimulation, the response was divided over the R_{baseline} (reflectance during frames 0-100), and an average of frames 150-300 was calculated.

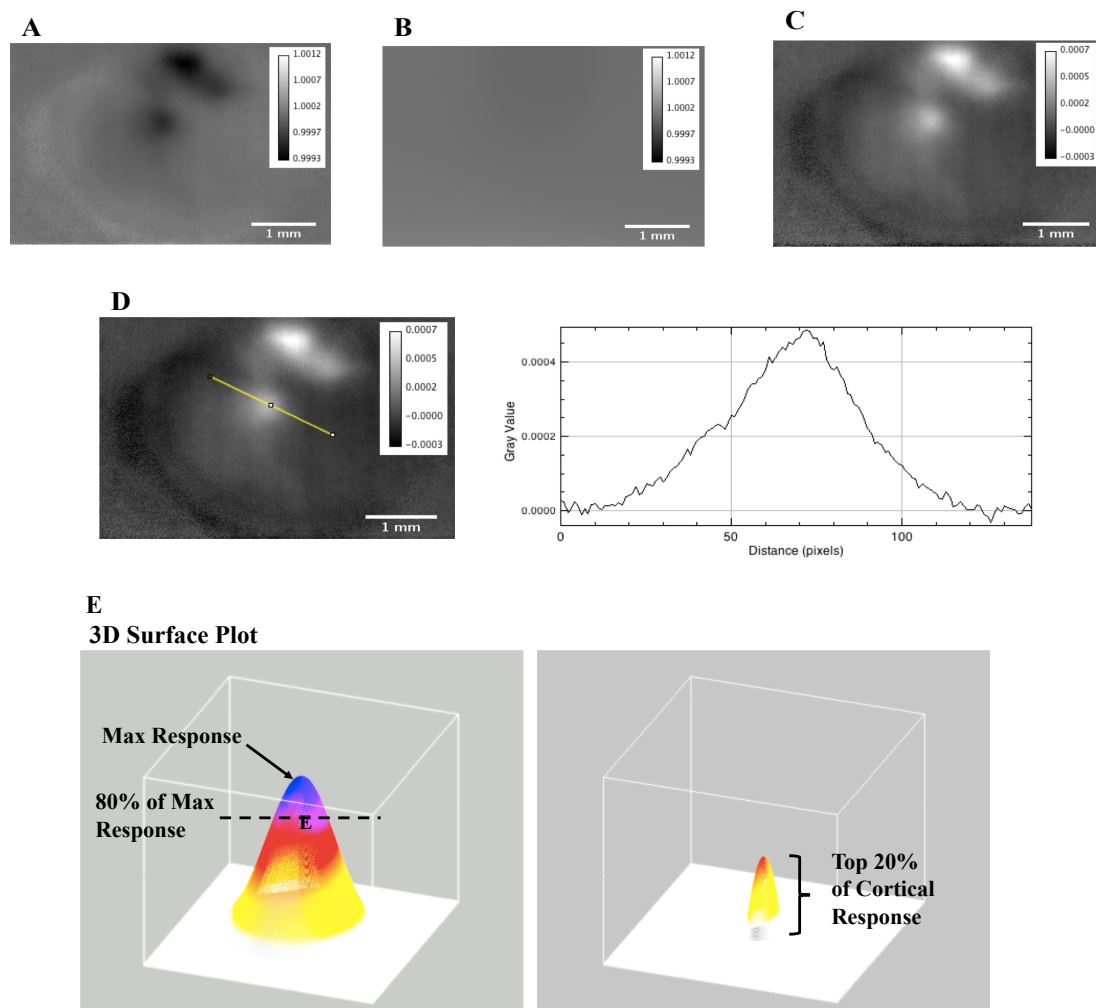


Figure 11, Measuring the fractional Changes in Reflectance (ΔR)

A) Shows the optical images averaged from 50 trials. Cortical activity is demonstrated as a black circle on the image. **B)** An average from frames 1 to 100 was calculated (baseline reflectance). **C)** Baseline reflectance subtracted from reflectance during 1 sec after stimulation onset (**B-A**). **D)** To measure the maximum value of response, a plot profile was graphed by drawing a line crossing the center of cortical response. **E)** The response was thresholded to 80% of that maximum value. The mean value of the pixels above this threshold was used to calculate the fractional changes in reflectance (ΔR).

2.3 Results

2.3.1 The level of long-term cortical plasticity due to whisker trimming is less in *Mecp2*^{-y} model mice compared to age-matched wild-type

To investigate the role of MECP2 in (experience-dependent) cortical plasticity of neuronal circuits in the barrel cortex, longitudinal IOS imaging in response to single whisker deflection was performed. The IOS was elicited in response to mechanical stimulation (20Hz for 1 Sec) of the contralateral C1 and B2 whiskers individually. Whisker C1 was trimmed to 3mm in length and kept short (3mm) for the next 14 days of the experiment. Whisker B2 was considered an untrimmed control whisker to exclude the possibility that symptom development due to *Mecp2* mutation could have affected the whisker deflection-evoked response of the barrel cortex. Pre-deprivation baseline imaging was acquired at day 0. IOS imaging was performed 7, 10, and 14 days after whisker trimming (Figure.12A). The result showed the amplitude response of barrel C1 was reduced over 14 days in both groups, (Figure.12B) whereas the response amplitude remained stable for barrel B2 (Figure.12C).

In wild-type animals (WT) (n=14), although the ΔR magnitude of B1 barrel was constant ($p=0.823$), a significant decrease in the magnitude of ΔR in C1 barrel cortex ($p=0.0000347$). Tukey's post hoc tests indicated a significant difference in ΔR between day 0 and 7 ($p=0.016$), day 0 and 10 ($p=0.0076$), and also day 0 and 14 ($p<0.0001$). This result suggested a presence of weakening of the C1 receptive field due to sensory deprivation, starting seven days after whisker trimming and persisting throughout the imaging period in a group of 4-6-week-old wild-type male mice (Figure.12D).

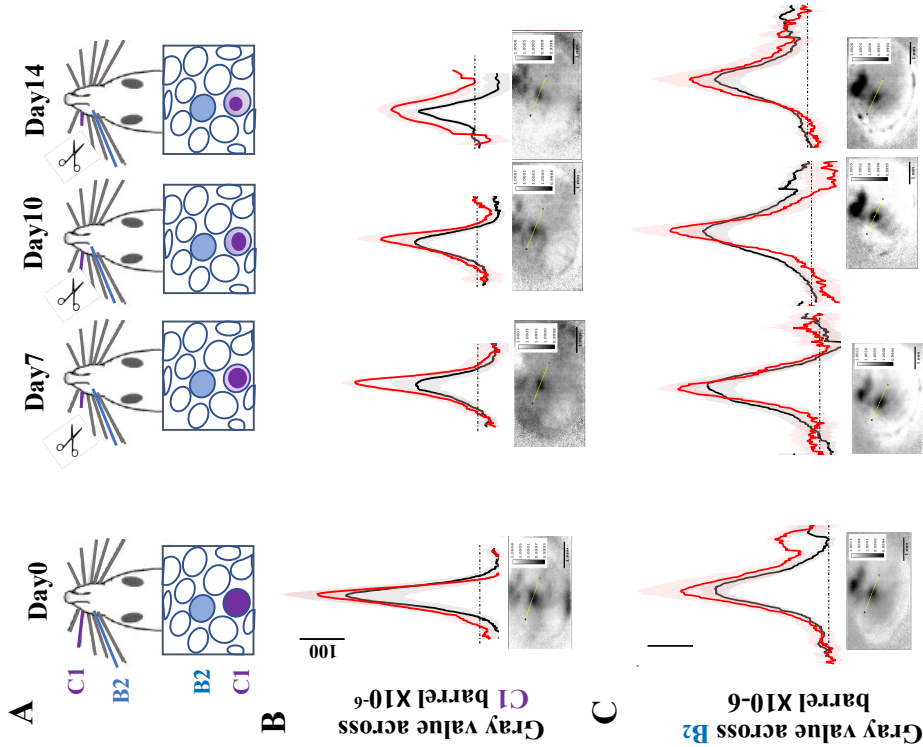
Data indicated a significant reduction of C1 receptive field response as a result of whisker deprivation over two weeks of experiments in Rett animals (RTT) (n=18) ($p=0.0417$). Since the amplitude of cortical evoked response (ΔR) of B2 barrel was unchanged ($p=0.648$),

the depth of anesthesia and the development of symptom does not appear to interfere with the cortical evoked response to the whisker stimulation. Tukey's post hoc tests showed the C1 barrel evoked response mean ($\pm$ SE) value differed significantly between day 0 and day 14 at $p=0.035$. However, there is no significant difference in the mean values between days 0 and 7 or day 0 and 10 or days 7 and 10. This indicated the rate of reduction in the cortical response as a result of sensory deprivation is slower in the RTT and is not detectable until day 14 of whisker trimming (Figure .12D).

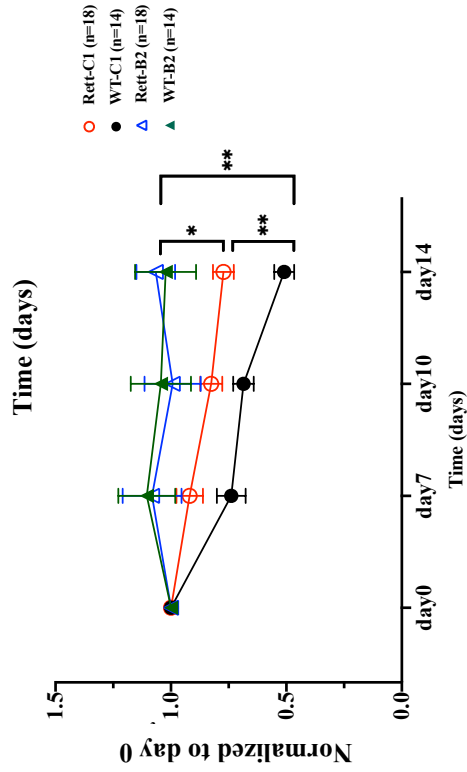
There were no significant differences in ΔR -value in either WT or RTT animals between day seven and day ten, showing that an additional three days of sensory deprivation does not affect the cortical response significantly. Comparing the value of response of both groups showed the initial response (pre- deprivation) in RTT was greater and stayed larger until day 14 (Figure.12C).

Normalizing the value of ΔR to baseline activity (ΔR on day 0) showed that in WT, at days 7, 10, and 14, the C1 barrel cortical response dropped by $26 \pm 6.2\%$, $31.37 \pm 4.4\%$ and $48.98 \pm 4.3\%$ respectively. In comparison, the amount of reduction of response in RTT was lower than that in WT ($8.05 \pm 5.7\%$ at day 7, $17.39 \pm 5.02\%$ at day 10 and $22.68 \pm 4.6\%$ at day 14). Although the overall reduction in the amplitude of the C1 barrel was observed in RTT, it was not significant until 14 days after whisker trimming. By day 14, reduction was only $22.68 \pm 4.6\%$ (from $3.4 \times 10^{-4} \pm 2.51 \times 10^{-5}$ on day 0 to $2.511 \times 10^{-4} \pm 2 \times 10^{-5}$ on day 14) in RTT which is close to the reduction in WT at 7 days of whisker trimming (from $2.6 \times 10^{-4} \pm 1.81 \times 10^{-5}$ at day 0 to $1.7 \times 10^{-4} \pm 1.7 \times 10^{-5}$ on day 10). Comparing the two genotypes showed a significantly bigger drop in C1 barrel response in WT after whisker trimming ($p=0.0135$). This difference was most noticeable on day 14 ($p<0.001$). During the 14 days of the experiment, we found a -50% change in C1 barrel response from the initial response (day 0) in WT compared to a -23%

change in RTT, while the evoked cortical response was sustained in both groups to the untrimmed whisker (B2) deflection ($p=0.750$) (Figure. 12E).



E



D

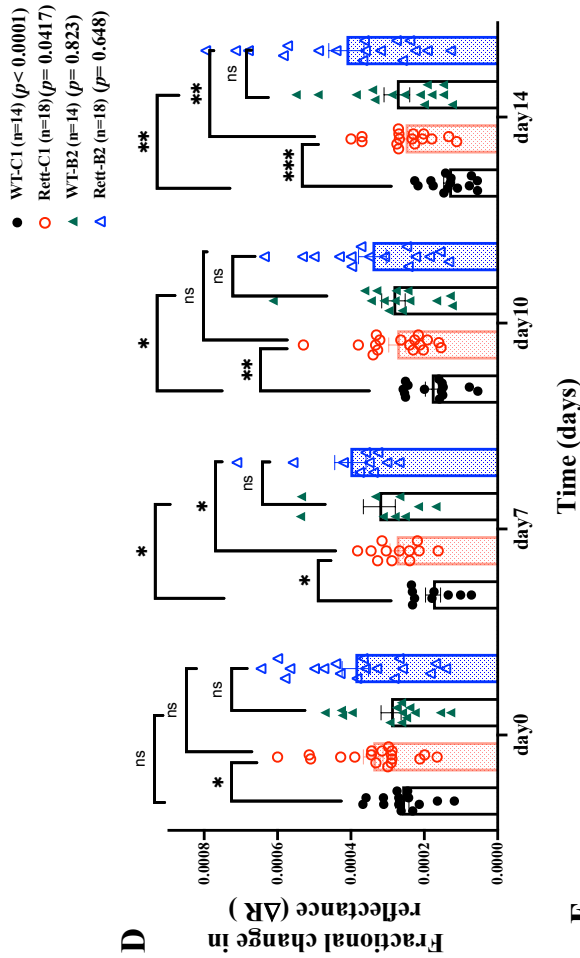


Figure 12, Whisker deprivation leads to a reduction in response of corresponding barrel cortex in 4-6 weeks old wild and Rett male mice. However, this reduction is less in the Rett group.

A) Schematic of experimental strategy and change in barrel response to a whisker trimming.

B) An Example of plot profile of an individual C1 barrel by drawing a line which is crossing from the maximum pixel value (wild-type group (WT) in black and Rett mice (RTT) in red). Amplitude of C1 barrel was reduced after whisker C1 trimming. Intrinsic Optical Signaling (IOS) images during the C1 whisker deflection before and after whisker trimming. The black spot on each image indicates the C1 whisker representation.

C) An example of the plot profile of particular receptive field corresponding to a stimulated whisker (B2) in WT (black) and RTT (red). The maximum amplitude response of the B2 receptive field was constant for 2 weeks.

D) Although the ΔR was stable for B2 barrel, it reduced due to whisker C1 deprivation in both groups. In WT animals, over 14 days of experiment, C1 barrel cortex response decreased significantly ($p < 0.0001$) due to whisker deprivation. This reduction started 7 days after whisker trimming and continued till day 14. In RTT, the overall decline in C1 barrel response following whisker trimming was observed ($p = 0.0417$). This reduction was significant on day 14.

E) Comparing the trend of cortical response reduction due to the whisker trimming in WT (black line) and RTT (red line) showed there was a significant difference in the degree of plasticity between these two groups ($p = 0.0135$). Although both groups showed the C1 barrel response reduction due to sensory deprivation, this drop in WT is more pronounced. By day 14, the C1 barrel response reduced to 50% of value in WT. However, it is only 24% in RTT. There are two big drops of response in the WT group, each about 26%, one from day 0 to day 7 and one from day 10 to day 14. This type of fast slop is not seen in RTT. In both groups, the response values of B2 barrel stayed almost the same for 14 days.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

2.3.2 Possible hypersensitivity in symptomatic male Rett model mice

Comparing the ΔR values on day0 showed the more prominent ($p=0.0281$) cortical response in RTT animals for the C1 barrel ($339 \times 10^{-6} \pm 26 \times 10^{-6}$) compared to the WT group ($260 \times 10^{-6} \pm 18 \times 10^{-6}$). The same pattern, but less pronounced, was seen for the B2 barrel cortex ($p=0.053$). Together this indicates the high impact of genotype on the size of the cortical response.

The IOS relies on blood oxygen saturation level (BOL), we measured it along with, heart rate, and breathing rate during the imaging session to investigate whether the physiological condition of RTT animals affect the magnitude of brain evoked response. While RTT mice are significantly lighter than WT ($p<0.0001$), the heart rate and breathing rate were similar (Figure 13). The BOL was significantly lower in RTT mice ($92.27 \pm 0.5\%$) than age-matched WT mice ($96.3 \pm 0.18\%$) ($p<0.0001$) (Figure. 13C). Since the BOL could interfere with the magnitude of brain evoked response collected with IOS, it appeared that lower oxygen level in RTT mice could be the cause of higher cortical response to the vibratory stimulation. However, there was no correlation between the magnitude of barrel response and BOL (Figure. 14), meaning the difference in the magnitude of cortical response is not due to the lower oxygen level in the RTT groups. In conclusion, RTT mice show hypersensitivity to the vibratory stimulation. This is consistent with a published study showing the hypersensitivity to a gentle touch in Rett model mice due to decreased inhibition of peripheral neurons (presynaptic) at the spinal cord dorsal horn (Orefice et al., 2016).

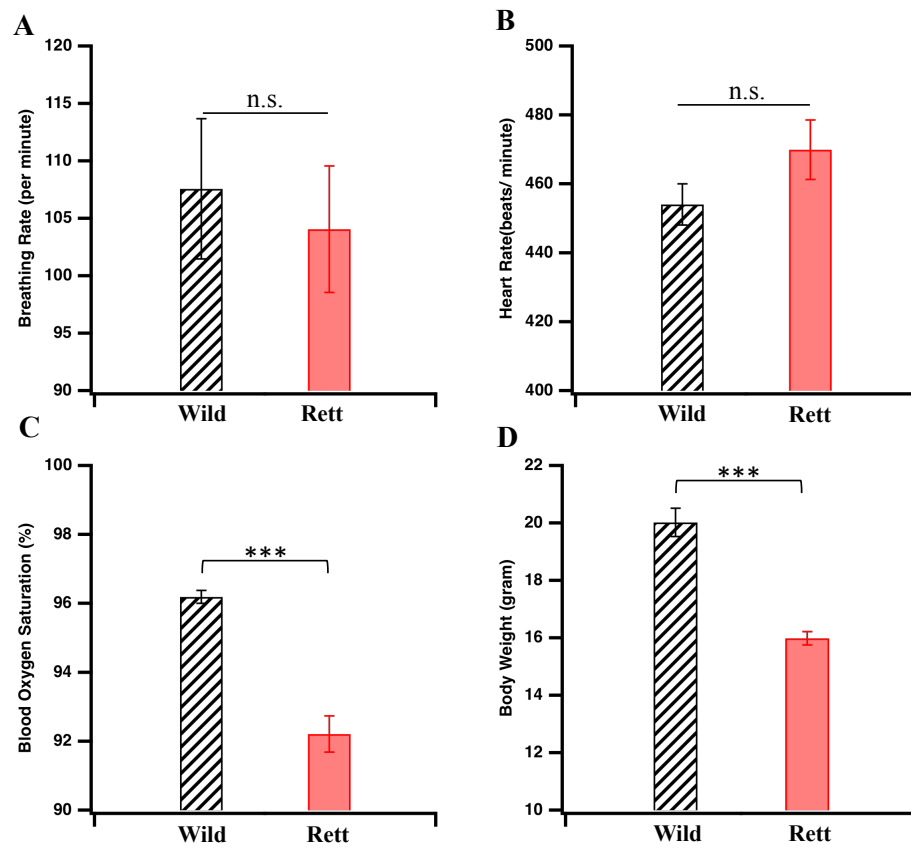


Figure 13, Physiological index in 2 months old male mice.

A, B) Heart rate and breathing rate were similar in Rett and wild-type groups. **C)** level of the blood oxygen saturation in Rett animals was significantly lower than wild-type animals ($p < 0.001$). **D)** Bodyweight was significantly smaller in Rett male mice than wild-type ($p < 0.001$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

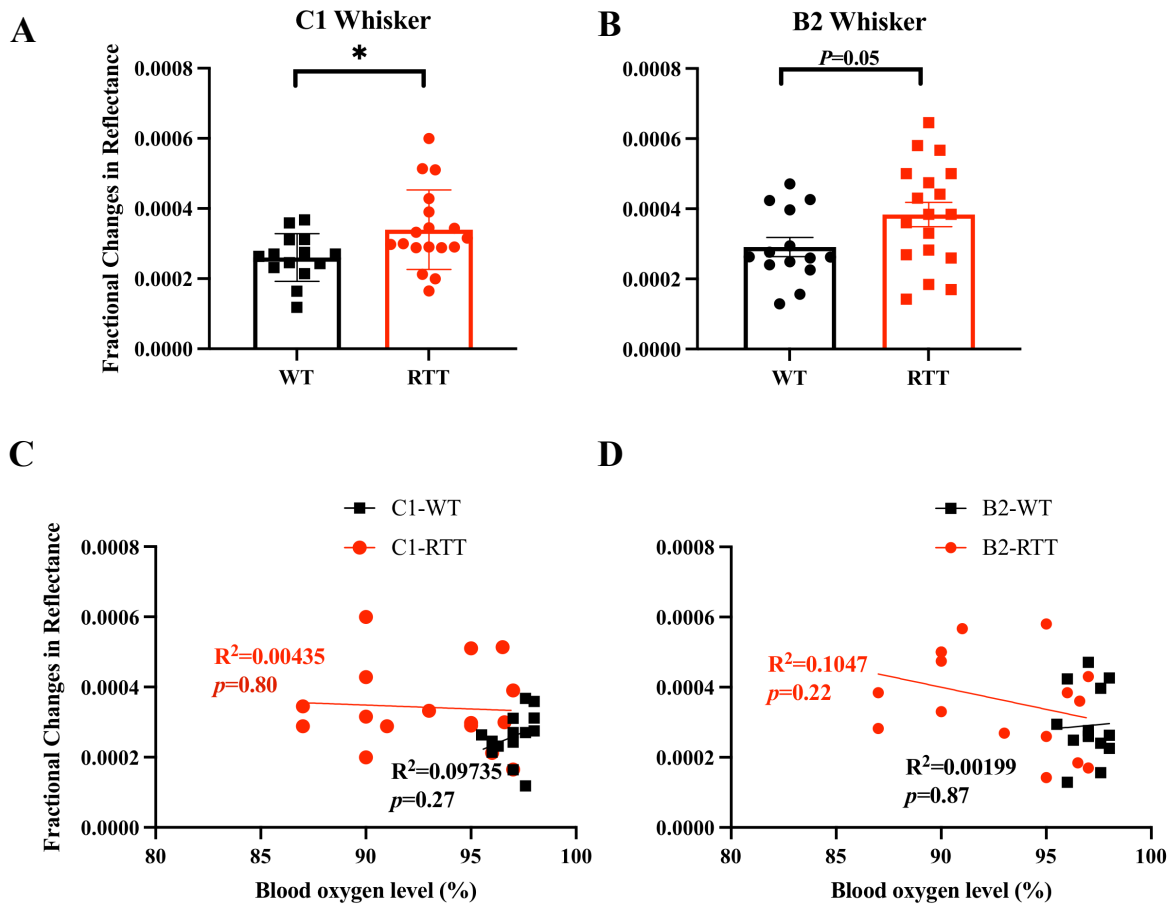


Figure 14, The effect of genotype on the magnitude of barrel cortex response to an identical external vibratory stimulation

A) Mean ($\pm$ SE) value of the C1 barrel cortex response to whisker stimulation showed a significantly larger response in RTT ($n=18$) compare to age-matched WT ($n=14$) ($p=0.0206$).

B) Response value (mean $\pm$ SE) of B2 barrel cortex to contralateral B2 whisker stimulation was higher in Rett (RTT) mice ($n=14$) in comparison to age-matched wild-type (WT) ($n=14$) ($p=0.053$).

There was no correlation between the magnitude of the C1 barrel (**C**) or B2 barrel (**D**) response to the blood oxygen level in the individual mice in either of the groups. $*p<0.05$, $**p<0.01$, $***p<0.001$

2.4 Discussion

2.4.1 Reduced long-term cortical plasticity in 4-6-week old *Mecp2*^{-y} mice

Our data implicate MECP2 as a critical player regulating long-term cortical plasticity. Inducing long-term cortical plasticity in 4-6-week-old male mice by trimming a whisker (C1) for two weeks indicated that both WT (n=14) and RTT (n=18) show a reduction in the barrel response while the response to wiggling the untrimmed whisker (B2) was unchanged in both groups. Plasticity is reduced in male RTT model mice in two ways. First, 14 days of whisker trimming led to about a 23% reduction in C1 barrel response in RTT, compared to 50% in WT. In addition, the amount of cortical response decline in RTT over 14 days was even smaller than the reduction in WT after seven days ($26 \pm 6.2\%$) of sensory deprivation. Overall, comparing the level of cortical plasticity between RTT mice and age-matched WT suggests a lower degree of long-term cortical plasticity over a particular time for RTT mice. Additionally, due to the slower rate of change in RTT mice, they require a longer time to show the same degree of cortical plasticity as age-matched wild-type show in a shorter time.

Similar to our finding, impaired experience-dependent plasticity has been shown in the visual (Tropea et al., 2009; Castro et al., 2014) and auditory (Krishnan et al., 2017) cortex of the Rett model mice. The cortical plasticity (ocular dominance plasticity) duration is extended to adulthood in female Rett model mice (*Mecp2*^{+/-}); while, it is observed only during a critical period of development in wild-type mice (Castro et al., 2014). Maternal experience changes the inhibitory circuit, which goes back to baseline after weaning in *Mecp2*^{+/-}, and not in wild-type, including increasing PV and PNN expression in the auditory cortex that alters the cortical plasticity (Krishnan et al., 2017).

Changes in IOS reflect the overall metabolic demand resulting from neural activity. With repeated whisker trimming, cortical layer 4 (L4) of the deprived barrel cortex does not

receive behaviorally generated (whisking) sensory input. After several days cortical responses observed by artificially wiggling the trimmed whisker are reduced. The reduced responsiveness is not the result of weakened synaptic input to L4 (Glazewski & Fox, 1996), instead there is a reduced activation of L2/3 by L4 due to a presynaptic form of LTD reflecting a decrease in probability of release from L4 to L2/3 (Allend et al., 2003; Bender et al., 2006). We observe that while whisker trimming does result in reduced activity in the deprived barrel of RTT mice, the reduction is about one half of that of WT mice. Impaired LTD as a result of *Mecp2* mutation has been observed in hippocampus, motor, and sensory regions of the neocortex (Asaka et al., 2006; Moretti et al., 2006; Li et al., 2016; Dani & Nelson, 2009). Although LTD in hippocampus is postsynaptic, impaired presynaptic LTD may be responsible for the blunted reduction in sensory deprived barrel cortex of RTT mice.

Cortical depression in barrel cortex resulting from whisker trimming is not a passive process; cortical activity is required (Fox, 2002). Blocking cortical activity by application of muscimol abolishes cortical depression following whisker trimming (Wallace et al., 2001). LTD in barrel cortex occurs when presynaptic action potentials and postsynaptic firing are not temporally coupled, for example if presynaptic action potentials arrive 100ms before postsynaptic firing (Feldman, 2000). L2/3 neurons above the deprived barrel do not receive sufficient input from the L4 underlying barrel; instead, they receive substantial input from neighboring barrels through inter-column neuronal connections (Wilbrecht et al., 2010). It is hypothesized that lack of input from deprived whisker causes the uncorrelation between L2/3 firing rate of deprived barrel and action potential from L2/3 of spread barrels. Thus, the random presynaptic activity will rise the number of unsynchronised postsynaptic depolarization and increases the LTD level in deprived barrel (Fox, 2000). Since the postsynaptic activity in deprived barrel can be controlled by spared barrel input and deprived barrel shows more depression if it is surrounded by more spared whisker (Fox, 2000), there are two factors that

could affect the LTD level in the deprived barrel; the cortical spontaneous activity and sensory input from neighboring spared whiskers to the deprived barrel. The lower LTD in RTT group might be due to the impairment of both or either of these factors. The lower cortical spontaneous activity in RTT mice has been reported (Dani et al., 2005; Asgarihafshejani et al., 2019; Zhang et al., 2020). Generally, the more often the postsynaptic fires the depression would be higher (Glazewski & Fox, 1996). Low spontaneous activity in RTT mice could potentially reduce the chance of unsynchronized firing between pre- and postsynaptic neurons and results in lower LTD. Additionally, possible reduced whisking in RTT group could provide less sensory input from spared barrels to the deprived one, causing a decline in LTD at deprived barrel because the depression will be lower if the deprived barrel is surrounded by columns that fire action potential rarely (Glazewski & Fox, 1996). Although we did not test the whisking rate in RTT in the current study, it could be examined in the future.

Additionally, PV⁺ interneurons have a crucial role in experience-dependent plasticity in the brain. There is a shift toward reducing PV⁺ interneurons' firing rate during learning due to a change in the number of excitatory and inhibitory boutons to these neurons (Donato et al., 2013). Change in the PV network configuration in *Mecp2* mutant mice interferes with experience-dependent plasticity in the brain and is associated with motor and learning impairment (Picard & Fagiolini, 2019). Loss of *Mecp2* causes increasing PV expression in both the sensory and motor cortex and enhances excitatory inputs onto PV⁺ interneurons (Morello et al., 2018). Increasing PV⁺ interneuron activity could be one reason for observed impairment in cortical plasticity in our data. While deletion of *Mecp2* from PV⁺ cells abolished experience-dependent cortical plasticity in the primary visual cortex during the critical period, loss of this gene in glutamatergic pyramidal cells or SOM⁺ did not have any effect on cortical plasticity (He et al., 2014). Since there is a complete lack of MECP2 in all the brain cells in our mice model, we are not able to conclude that the observed cortical plasticity deficit is only due to

the change in the level of PV⁺ interneurons activity in the cortex. Abnormality in cortical PV⁺ interneuron activity in Rett model mice could be one of the factors that affect the cortical plasticity impairment in this group, but it is not the only one.

2.4.2 Conclusion

Our data indicate impairment of long-term cortical plasticity due to sensory deprivation in 2-month-old RTT male model mice. However, it does not show whether the observed abnormality is due to the role of *Mecp2* in Hebbian/hemostatic plasticity or if it is a result of altered cortical inhibitory circuits in Rett animals' brains. The reduced long-term plasticity in Rett male animals could be an explanation of learning and cognitive deficits in RTT patients. Understanding circuit alterations that underlie behavior deficits is a key to identify the pathogenesis of RTT, which could provide the possibility of developing new strategies to improve the RTT patients' quality of life.

Table 1: statistical analysis of chapter 2 result

Figure	statistical test	values
Fig 12 D	*RM One-way Anova	C1-WT: $F(3,47) = 9.9322, p = 0.0000347$ C1-RTT: $F(3,59) = 2.914, p = 0.0417$ B2-WT: $F(3,47) = 0.302, p = 0.823$ B2-RTT: $F(3,56) = 0.552, p = 0.648$
E	RM Two-way Anova	C1 & B2-WT: $F(3,71) = 5.231, p = 0.0026$ C1 & B2-RTT: $F(3,85) = 2.845, p = 0.042$ WT & RTT-C1: $F(3,76) = 5.897, p = 0.0011$ WT & RTT-B2: $F(3,80) = 0.1402, p = 0.93$
Fig 13 C D	2 tailed-Unpaired t-test	$t = 1.98, df = 30, p < 0.001$ $t = 2.04, df = 124, p < 0.001$
Fig 14 A B	2 tailed-Unpaired t-test	$t = 2.308, df = 30, p = 0.0281$ $t = 2.008, df = 30, p = 0.053$

For all the figures, the sample size (n) is: RTT=18, WT=14

*RM: Repeated measured

3 Persistent sensory adaptation due to continuous hindlimb stimulation is altered in Rett model mice

3.1 Introduction

3.1.1 Overview

The aim of the chapter was to investigate the cortical plasticity by examining sensory adaptation in Rett model mice. We applied brief and sustained vibratory stimulation to the hindlimb (HL) to Rett model mice and age-matched controls and recorded the evoked cortical activity from primary HL somatosensory cortex using Intrinsic Optical Signaling (IOS) imaging and also Local Field Potential (LFP) recording.

3.1.2 Somatosensory nervous system

We constantly receive sensory information from the environment through our sensory organs. Aristotle classified touch, vision, hearing, smell, and taste as the five primary senses (Abraira & Ginty, 2013). Sensory receptors turn the energy of external stimuli into electrical sensory signals (sensory transduction). A sensory receptor can be a specialized structure at the end of a peripheral nerve or a separate cell that communicates through a synapse to an afferent neuron which transfers signals to the central nervous system (CNS). In the nerve ending, a stimulus increases the cation channel open probability to produce a depolarizing current, which, if large enough, generates action potentials in the afferent axon.

In the current project, we focused on the somatosensory system (touch and proprioception properties). The somatosensory system provides three functions; exteroceptive and interoceptive that provide information about external and internal (visceral) stimuli,

respectively, and proprioceptive that supplies information about the position of the body needed to maintain balance or execute movements (Abraira & Ginty, 2013).

Different types of afferents link somatosensory receptors to the CNS divided into groups based on their axon diameter and the degree of myelination. The A group of fibers, which are myelinated by Schwann cells, are up to 10 times larger in diameter than C fibers. C fibers are the most abundant and but their thin diameter (0.2-1.5 μm) and lack of myelination results in a slow conduction velocity of 0.4-2 m/s. C fibers are grouped into Remak bundles (Rich & Brown, 2018). C and $A\delta$ afferents conduct pain, temperature, and itch signals to CNS. $A\delta$ fibers are lightly myelinated (1-5 μm axonal diameter) with a conduction velocity of 4-26m/s afferents. $A\beta$ afferents (6-12 μm diameter) convey tactile, touch information (Floden & Combs, 2012). They are wrapped with myelin sheets that are less than the myelin sheet although these are not as thick as those around the larger 13-20 μm diameter $A\alpha$ afferents, which convey information related to proprioception (muscle sense). As a result the conduction velocity of $A\alpha$ afferents (72-120m/s) is almost double that of the $A\beta$ afferents (46-72 m/s) (Brown & Iggo, 1967). Type I and II afferents, with the same diameter and conduction velocity as $A\alpha$ and $A\beta$ afferents, convey signals from muscle spindle and Golgi tendon organ of muscles (proprioception). In the current project, the amplitude of applied vibration is low so we expected that predominantly $A\beta$ afferents are activated.

Mammalian skin can be divided into hairy and non-hairy (glabrous) skin. Skin mechanoreceptors ($A\beta$ afferents) are specialized to receive tactile information. There are various kinds of mechanoreceptors in each skin type: Pacinian corpuscles, Ruffini endings, Meissner corpuscles, and Merkel's discs (Johansson & Vallbo, 1983) (Figure .15). In hairy skin, hair follicles transduce tactile stimuli. There are three types of hair follicles (guard, awl/auchenne, and zigzag) in mice (Li et al., 2011). Details about receptors in hairy skin will

not be described further as the mechanical stimulation as a vibratory stimulus applied to the distal hindlimb (HL) via a rod glued to the skin likely does not stimulate hair follicles substantially.

Mechanoreceptors in the glabrous skin are either slowly adapting receptors (Ruffini endings and Merkel's discs) or rapidly adapting receptors (Pacinian corpuscle and Meissner corpuscle), based on the temporal dynamics of their response to sensory stimulation. Rapidly adapting receptors (RA), fire action potential only in the beginning or at the end of mechanical stimulation, while slowly adaptation fire action potential even during the presence of mechanical stimulation (Coleman et al., 2001). They can also be divided into two groups based on their receptive field size; small receptive field (Merkel and Meissner corpuscle) and large receptive field (Ruffini endings and Pacinian corpuscle). Given that the mechanical stimulus was primarily vibratory we conclude most of the sensory signals transmitted to the CNS were the result of activation Pacinian corpuscles, Meissner corpuscles and to some extent, joint receptors.

In humans, Pacinian corpuscles, located deep in the dermis, can respond to vibrational stimuli as high as 500Hz and can fire for each vibratory cycle (Kandel et al., 2013). This mechanoreceptor comprises an individual A β afferent. The nerve ending is wrapped in an onion-like sheath formed by connective tissue (lamellar cells) (Nikolaev et al., 2020). In Meissner corpuscles, the nerve terminal is sandwiched between two or more lamellar cells. Pressure on lamellar cells causes the opening of Na⁺ channels on the nerve ending to generate the action potential. In mice, typical encapsulated Pacinian corpuscles and Meissner corpuscles are located in the vicinity of the periosteum of the distal part of the fibula under the crural interosseous membrane and toe and finger pads, respectively (Albuerne et al., 2000).

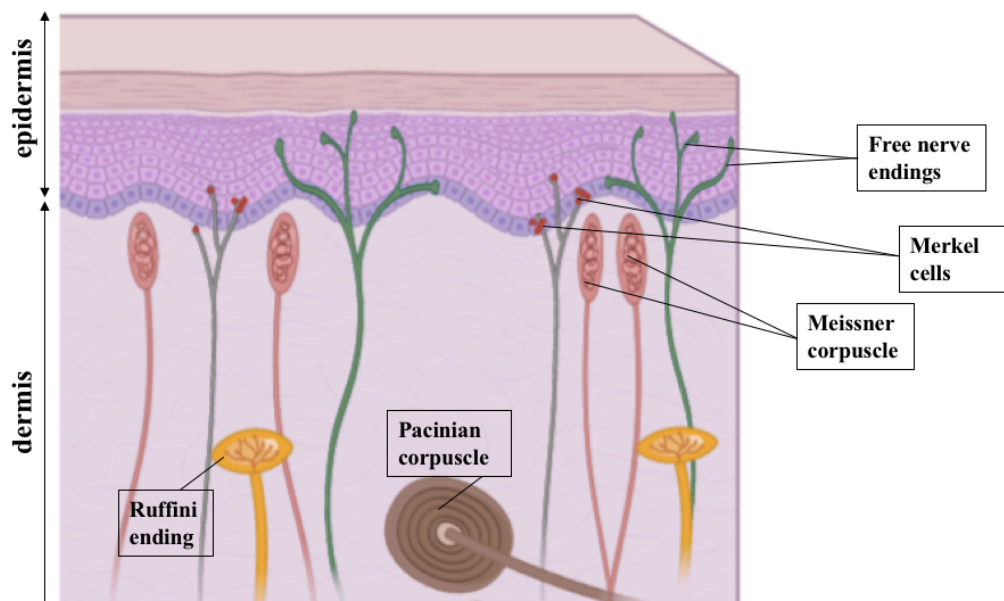


Figure 15, The arrangement of 4 types of cutaneous mechanoreceptors in mammalian skin

Merkel cells/neurite complex are located in the basal layer of epidermis. They are excitable cells, and each cell makes a synapse-like association with a single A β afferents. They help us to distinguish the shape and texture of objects. Meissner corpuscles, formed by a connective tissue capsule that includes lamella of Schwann cells, are located in the dermal papillae. They are sensitive to low-frequency vibration, detect movements on the skin, and help us to control grip. Ruffini endings found deep in skin are elongated, spindle-shaped, capsular specializations. They detect limb movement and skin stretch. Pacinian corpuscle looks like an onion surrounding a single A β afferents and located deep in the dermis. They respond to skin movement and skin vibration. (Modified from (Abraira & Ginty, 2013))

Shaking the foot due to the vibratory stimulus could activate the joint capsule receptors. Joint capsule receptors, which respond to joint movement, are a group of mechanoreceptors located in joints, along with muscle spindles and Golgi tendon organs are involved in proprioception for motor control. Joint receptors have features similar to Ruffini terminals and

Pacinian corpuscles. For example, Pacinian corpuscles in the joint capsule are smaller than those in the dermis and are innervated by medium-diameter ($A\beta$) fibers.

3.1.3 Somatosensory pathway signaling

They are three types of neurons involved in signaling, from sensory receptors (in this case, mechanoreceptors in the HL) to the somatosensory cortex (Figure. 16).

Primary neurons (primary afferent fiber) are pseudo-unipolar with soma located in the dorsal root ganglion. They transmit the sensory inputs to the spinal cord. The axon of dorsal root ganglion neurons has two branches; one branch projects to the periphery, the other projects to the CNS.

Primary neurons synapse with secondary fiber in the brainstem (medulla). The afferents from the lower limb synapse in the gracile nucleus and those from the upper limb to the cuneate nucleus. The secondary fibers, which project from the medulla, cross the spinal cord and terminate in the ventral posterior lateral nucleus (VPL) of the thalamus via the dorsal column medial lemniscal column system.

Thalamic relay cells provide excitatory input to the primary somatosensory cortex (S1). With many of the thalamic afferents project to layer 4 (L4) of the somatosensory cortex onto both excitatory and inhibitory neurons (Figure. 16).

Sensory information is processed in a feedforward manner from the periphery to the cortex, but there are also extensive feedback projections to the thalamus (Figure 17). In the thalamocortical circuit, there are feedback projections from the cortex (L5/6) to the thalamus directly and also through the thalamic reticular nucleus (TRN) (Whitmire & Stanley, 2016). TRN is a thin structure surrounding the dorsal thalamus that contains predominantly inhibitory neurons (Houser et al., 1980). In rodents, it provides inhibition to all thalamic nuclei (Arcelli

et al., 1997). TRN receives input from the primary and secondary somatosensory cortex (S2) and also from the thalamus. Neurons within the TRN are inter-connected by GABAergic synapses. The inhibitory neurons in TRN include PV⁺ and SOM⁺ (Clemente-Perez et al., 2017).

The thalamus integrates the ascending driver inputs from the brainstem (raw sensory information) with descending signals from L5/6 and transfers the integrated sensory information back to the brain (Groh et al., 2014).

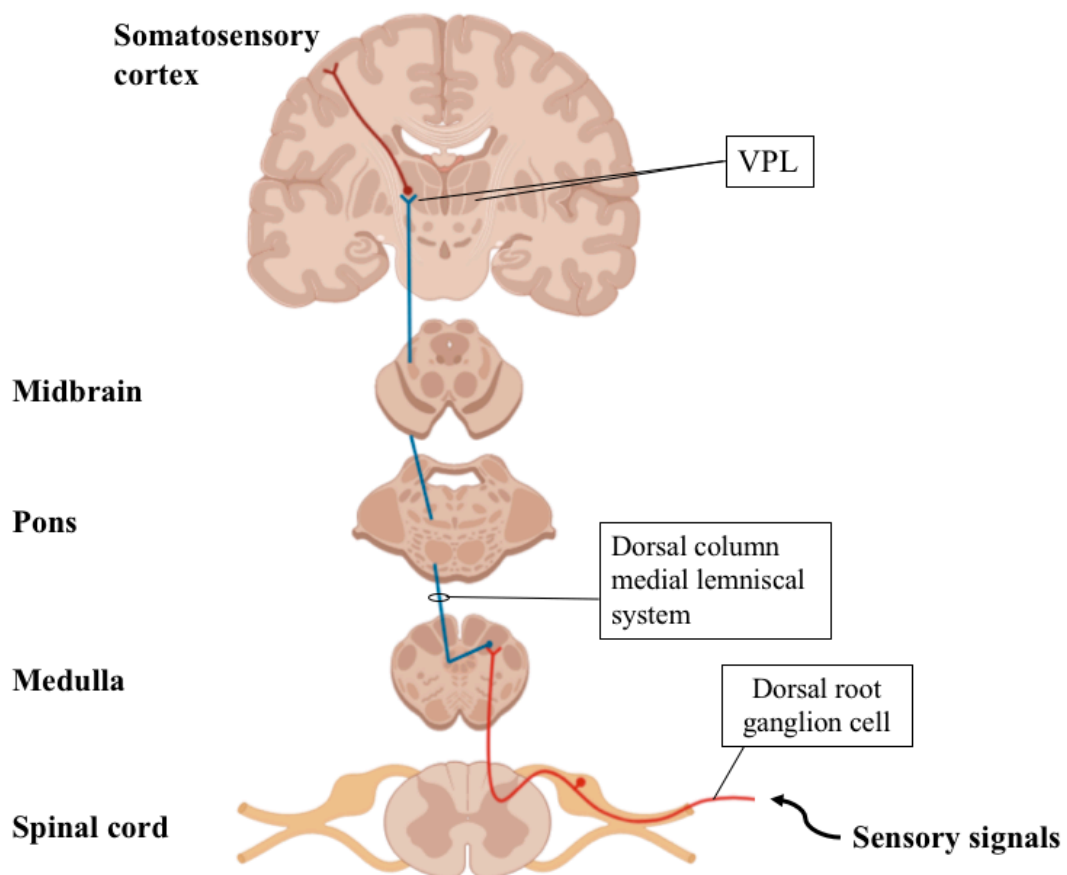


Figure 16, Hindlimb to primary somatosensory pathway

Sensory signals, including tactile and vibratory information, transmit to the spinal cord through dorsal ganglion cells, primary somatic afferents. Dorsal ganglion cells synapse with secondary neurons in the brainstem (medulla), form the dorsal column. Secondary neurons synapse with third-order neurons in the thalamus (VPL). From the thalamus, sensory signals project largely to layer 4 of the corresponding area of somatosensory cortex.

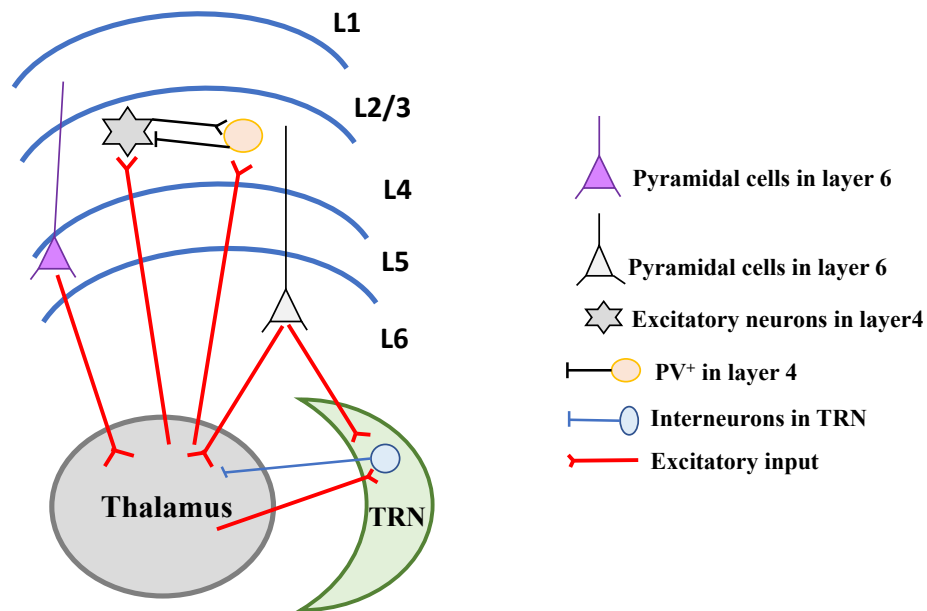


Figure 17, Sensory information is processed in a feedforward and feedback manner from the periphery to the somatosensory cortex.

A majority of thalamic excitatory inputs project to layer 4 (L4) of somatosensory cortex onto both excitatory and inhibitory neurons (feedforward). Pyramidal neurons from layer 6 and 5 (L5, L6) send excitatory inputs to the thalamus and thalamic reticular nucleus (TRN). TRN receives sensory inputs from cortex and thalamus and sends inhibitory inputs to the thalamus (feedback).

3.1.4 Sciatic Nerve

In the final part of this chapter, compound sensory nerve action potentials (SNAPs) of the sciatic nerve were recorded to investigate whether the sensory inputs to the spinal cord were reduced due to the continuous vibrational stimulus on mice HL. The sciatic nerve is the largest in both humans and mice, running from the lower back through the back of the legs. The mouse sciatic nerve originates predominantly from the third and fourth lumbar spinal nerves (a mouse has a total of six lumbar vertebrae) (Rigauda et al., 2014). It divides into sural, tibial, and peroneal branches at the popliteal fossa. The sural nerve includes some axons that innervate thin glabrous skin on the lateral edge of the HL (Smith et al., 2013). The tibial nerve innervates the hind paw glabrous skin (Walcher et al., 2018). Tibial nerves contain A β mechanoreceptors, A δ , and C fibers. 40% of A δ fibers in the tibial nerve are mechanosensitive (Cain et al., 2001).

3.1.5 Adaptation

Adaptation is a reduction of neuronal responses in the presence of repetitive stimulation. In many sensory synapses, a decrease in neuronal responses to the presence of repetitive stimulation has been seen. When we refer to adaptation in a sensory network, this potentially includes change in intrinsic neuronal properties of receptors or interneurons and/or change in synaptic transmission.

Adaptation at the level of a neuron's intrinsic properties usually means that the rate of spiking in a neuron is reduced in response to a sustained input. Somatic currents are involved in the change of firing rate in a particular neuron. Adaptation can be produced by among other processes M-currents (Constanti & Brown, 1981), Ca²⁺ activated and Na⁺ activated potassium currents (Bhattacharjee & Kaczmarek, 2005) and slow recovery from the activation of voltage gated Na⁺ channels. All these mechanisms can cause a change in the firing rate of a particular

neuron in an activity/cell type manner (Whitmire & Stanley, 2016). Neurons do not work in isolation. As part of a larger network, a small change in an individual neuron's activity pattern can have a large consequence for the output on the whole network.

Adaptation could also occur at the synaptic level. Synaptic efficacy (the capacity of presynaptic input to influence postsynaptic output) could be modulated due to continuous stimulation. In general, the postsynaptic potential could be reduced (depression) (Regehr, 2012) or enhanced (facilitation) due to repeated spiking activity of the presynaptic neuron.

In this chapter, we studied short-term adaptation (STA) that develops during a short train of 7 stimuli and a form of longer lasting adaptation which we call persistent adaptation (PA) that is seen as a decrease in the evoked cortical response lasting an hour or more following many minutes of repeated sensory stimulation.

3.2 Experimental Rationale

Disruptions of somatosensory pathways have been reported in some RTT patients and Rett model mice, but the location and characteristics of circuit alterations are not clear (Nomura et al., 1987; Bader et al., 1989; Kálmánchey, 1990; Yoshikawa et al., 1991; D'Cruz et al., 2010; Goffin et al., 2012; Liao et al., 2012). In addition, RTT is characterized by cognitive and motor impairments (Dolce et al., 2013; Adcock et al., 2020). Cognitive impairments include deficits in learning, memory, and sensory information processing, phenomena which invariably require some form of synaptic plasticity. Additionally, the motor deficits might be due to impaired sensory inputs or sensory processing. As a way of exploring possible altered neural function and plasticity in the brain of Rett model mice, we conducted studies of a simple form of plasticity, sensory adaptation, in the limb somatosensory pathway. The evoked brain response was recorded from the corresponding somatosensory cortex with Intrinsic Optical Signaling (IOS) imaging and intracortical Local Field Potential (LFP) recording.

We studied sensory adaptation in pre-symptomatic and symptomatic female Rett model mice in addition to null male mice. We began by studying the sensory adaptation in male Rett model mice. As mentioned in the previous chapter, the absence of any functional MECP2 protein in the deficient males group results in observing a more apparent abnormalities so we surmised that if adaptation were affected it should be most clearly seen in these mice. However, in humans RTT predominantly affects females as it derives almost exclusively as the result of *de novo* mutations in the father's sperm (Trappe et al., 2001). Although, female Rett model mice (*Mecp2*^{+/-}) derive their mutated X from their heterozygous mother, since male nulls are not capable to breed they are still considered more clinically relevant the female RTT patient population. Consequently, we applied the same sensory adaptation protocol to female Rett model mice. Based on the fact that symptom development in both female RTT humans and mice occurs many months after birth we studied sensory adaptation in both pre-symptomatic (2-month old) and highly symptomatic (10-12-month old) mice to address whether altered sensory processing could be observed prior to the appearance of overt motor symptoms.

Sensory adaptation was measured using Intrinsic Optical Signaling (IOS) imaging and intracortical Local Field Potential (LFP) recording. IOS is a noninvasive technique that enables recording the brain activity from an intact mouse brain through a scale. However, it has poor temporal resolution and relies on activity dependent changes in the level of blood oxygen (Frostig & Chen-Bee, 2009), and Rett model mice (especially null male mice) have breathing deficits (Ogier & Katz, 2008). Therefore, LFP, a non-optical method with higher temporal resolution (Gabriel et al., 2018) was also used to assess sensory cortical activity.

The neuronal activity related to the processing of particular stimulus can be examined by averaging evoked potentials (Troncoso et al., 2000). An evoked potential is obtained by averaging multiple EEG segments aligned to the onset of the stimulus and allows estimation

of the time course of the brain response to a stimulus on a background of spontaneous neural activity (Sysoeva et al., 2020).

To study cortical plasticity, evoked brain activity was recorded with short trains of tactile stimuli applied before, during and after a period of sustained HL vibration. A decline in evoked responses was seen to develop as the period of sustained vibration was extended and this reduced response persisted for more than an hour afterwards in wild-type mice. To control for possible changes in the response of the primary afferent endings compound sensory nerve action potentials (SNAPs) of the sciatic nerve were analyzed. The sciatic nerve is the most frequently chosen nerve in peripheral nerve-related studies (Nakamura et al., 2001; Ma & Eisenach, 2003; Bala et al., 2014; Rich & Brown, 2018) since it is the largest nerve and easily accessible in mice. Also, in most animals, the fundamental anatomy of the sciatic nerve is similar. For this study, the SNAPs of the sciatic nerve were recorded *in vivo* during PA and recovery phases to indicate whether the observed sensory adaptation is central or peripheral.

3.3 Methods

3.3.1 Animal

Mice with deficient in the *Mecp2* gene were bred from heterozygous females (*Mecp2*^{+/-}) with wild-type (C57BL/6) males. The heterozygous Rett females (*Mecp2*^{+/-}) were provided from UC Davis MMRRC (*Mecp2*^{tm1.13ac}). They lack exon three on the *Mecp2* gene. 4 to 6 weeks old male, 10 to 12 months old female (symptomatic), and 2-month-old female (pre-symptomatic) animals were used for these sets of experiments. Control groups include age-matched wild type mice (C57BL/6). All mice were housed in 12h/12h dark/light cycles. PCR was performed to approve the animal's genotype.

3.3.2 Evoked Response Recording with Intrinsic Optical Signaling (IOS) Imaging

3.3.2.1 Surgery

During surgeries, each animal was anesthetized with 1.5% isoflurane:air mixed. The depth of anesthesia was checked by the response to a toe pinch. During the surgery, body temperature was measured by a rectal probe and maintained between 36.5°C and 37°C by a thermostatically controlled heating pad. A circular region of the skull over the somatosensory cortex (right hemisphere) was cleaned, and a thin layer of clear dental cement (C&B - Metabond) was applied over the exposed (intact) skull with a 3-mm diameter coverslip placed on the cement. Once the dental cement dries, surface vessels are clearly visible through the intact skull. The cranial window was applied to the skull three days prior to the imaging sessions.

3.3.2.2 Hindlimb stimulation

To generate brain activity corresponding to hindlimb (HL) stimulation in IOS experiments, vibratory stimulation at 100Hz was applied for 1 second to the left HL with a piezoelectric wafer (model number). A 2.5 cm long pencil lead was attached to the end of the piezoelectric device with the other end glued between toe 3 and 4 on the top side of the paw. Baseline IOS responses to 1 second of 100Hz vibration were obtained as described in section 1.3.2.2. After establishing the baseline response, the HL was stimulated for a total of one hour (100Hz, 1sec, ISI 6 sec), with vibration evoked IOS tested every 15 minutes following a 2-minute rest period (Figure. 18). Upon completion of the sustained stimulation period the response to 100 Hz, 1 second test stimuli were obtained at 15-minute intervals over the next hour.

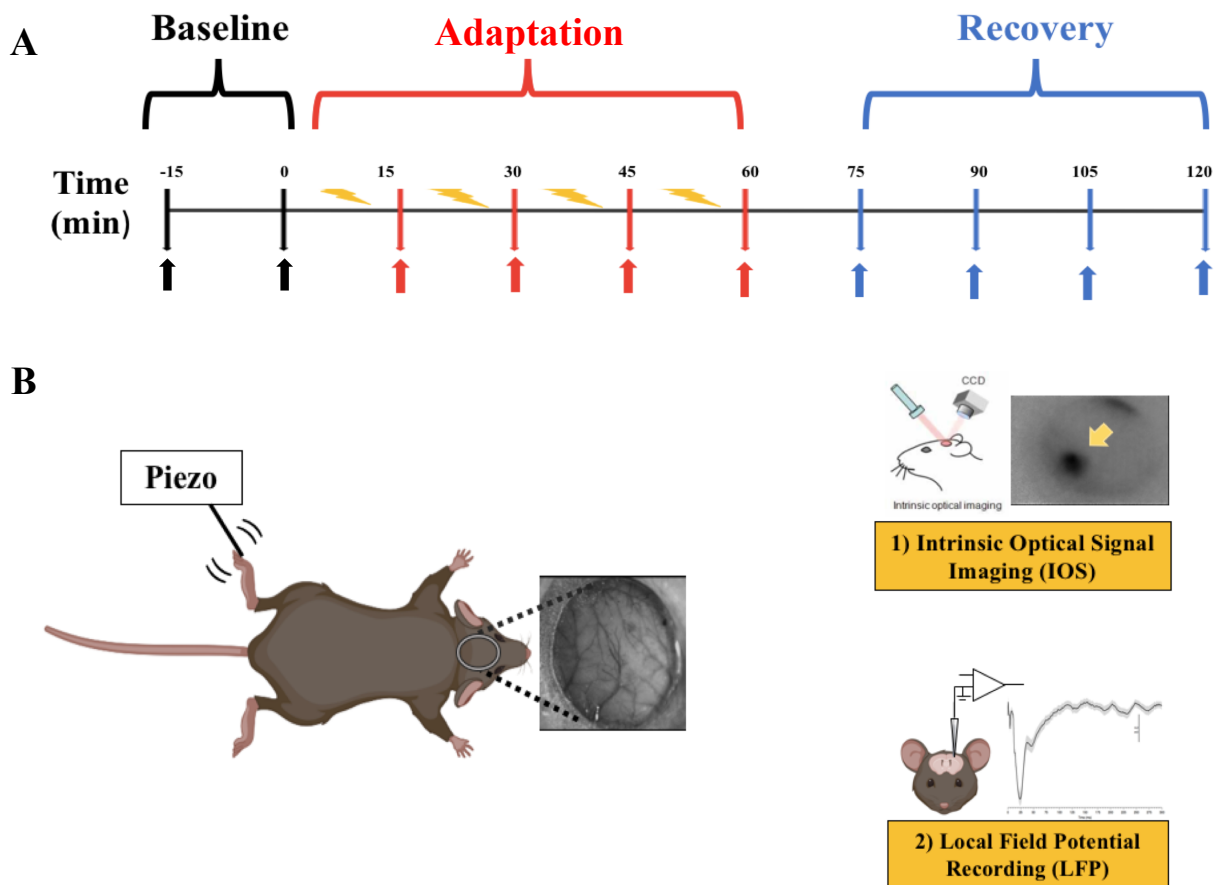


Figure 18, Sensory adaptation protocol

A) Shows the sensory adaptation protocol. Brain baseline activity was recorded two times with 15 minutes intervals (black arrows) prior to the adaptation phase. To induce sensory adaptation (PA), a vibration stimulation was applied to the hindlimb for 1-hour at 100Hz for one second every 6 seconds. During the adaptation phase, brain activity was recorded every 15 minutes (red arrows). Recovery was monitored at 15 min intervals for 1 hour after the adaptation phase (blue arrows).

B) A cranial window was implanted on top of the somatosensory cortex on the right hemisphere. The left hindlimb was stimulated with a piezoelectric for 1 sec at 100Hz. Cortical activity was recorded with Intrinsic Optical Signaling (IOS) imaging and Local Field Potential (LFP) Recording.

3.3.2.3 IOS Imaging

During the imaging sessions, animals were anesthetized with 1% isoflurane:air. The heart rate, breathing rate, and blood oxygen saturation were monitored with an oximeter during the imaging sessions. Brain activity was collected with IOS for 3 seconds (1 second before stimulus onset, 1 second during the stimulus, and 1 second of brain activity after stimulus termination). Stimulus trials were repeated five times with a 14-second inter-trial interval. Acquisition of brain reflectance values and image processing were performed as described in the previous chapter. Fractional reflectance changes, ΔR were plotted as mean $\pm$ SE.

3.3.3 Electrophysiology (Local Field Potential recording)

3.3.3.1 Surgery

1-2 days prior to electrophysiological recording the somatosensory cortex corresponding to HL stimulation was mapped with IOS imaging to determine subsequent electrode placement. During the surgery, each animal was anesthetized with 1.5% isoflurane: air, and the depth of anesthesia confirmed by lack of response to a toe pinch. Body temperature was measured by a rectal probe and maintained between 36.5°C and 37°C by a thermostatically controlled heating pad.

To locate a reference electrode, a 1.5mm diameter depression was drilled to just above the cranial surface over the left posterior hemisphere of the cerebral cortex (coordinate: bregma -4.16mm lateral 1.5mm). A 0.005'0.007" Teflon™ coated Ag-AGCl reference electrode, was inserted about 0.5 mm deep into the depression and secured with cyanoacrylate glue. The skull on over the right HL primary somatosensory cortex (previously determined by IOS) was thinned by a dental drill until small cracks appeared in the bone sufficient to allow insertion of the recording electrode. A borosilicate glass capillary (O.D.=1.5mm, I.D.=0.86mm, Sutter Instruments) pulled to a $\approx 2.5\mu\text{m}$ tip diameter with 1.5M Ω resistance, filled with brain buffer

(NaCl 135mM, KCl 5.4mM, HEPES-Na salt 5mM, MgCl₂ 0.5M, CaCl₂ 0.5M, PH=7.35) was inserted to a 350µm depth from the pial surface in the somatosensory cortex (center of HL corresponding area) as a recording electrode. An Ag-AgCl wire was located under the skin at the back of the mouse head and glued to the skin as a ground electrode (Figure. 19).

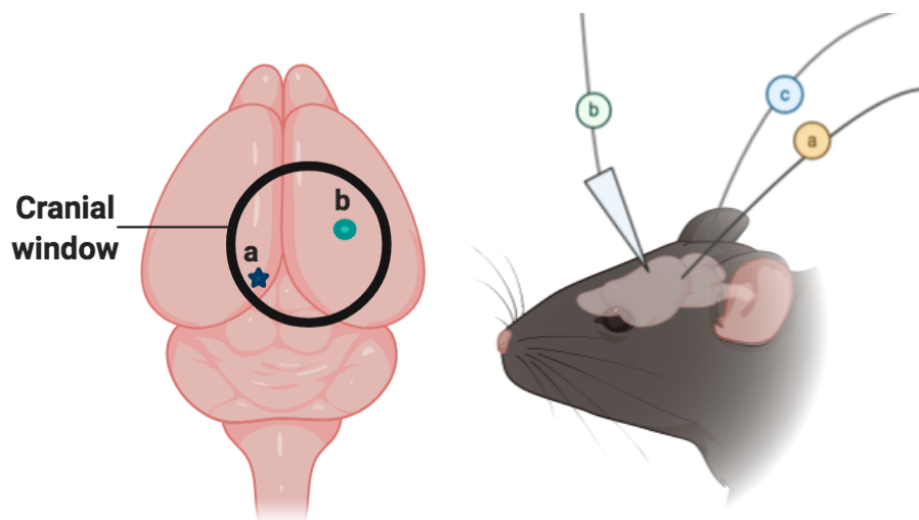


Figure 19, Location of electrodes to record local field potential (LFP) activity of a brain

A reference electrode was inserted about 0.5mm into the posterior region of the brain in the left hemisphere (a). A recording electrode (a glass micropipette) was inserted to the center of the hindlimb receptive field (300-350 µm below the surface) on the somatosensory cortex at the right hemisphere (b). Animals were grounded with an electrode located under the skin at the back of the head (c).

3.3.3.2 Depth of Recording

To determine the best depth (maximum response) for recording the brain activity, a recording electrode was inserted through the center of the HL receptive field, and the brain response to HL stimulation recorded at different depths (HL receptive field was mapped by IOS in advance) (Figure. 20A). The maximum response amplitude was recorded at 300-400 μm depth from the pial surface (Figure. 20B). Therefore, local field potential recording was obtained from an electrode positioned at 350 μm below the brain surface in all experimental animals.

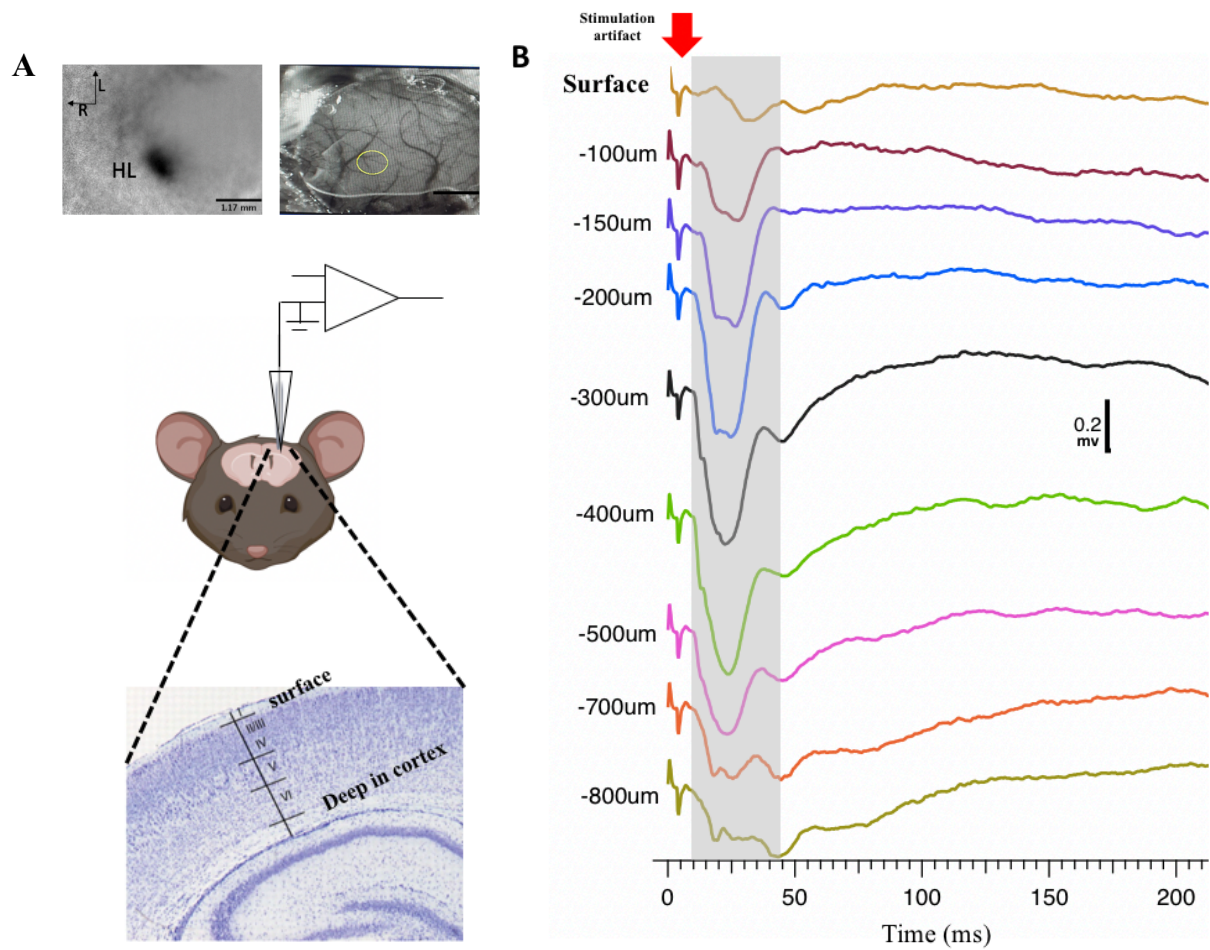


Figure 20, The maximum response amplitude was measured at 300-400 μm depth below the brain surface

A) A recording electrode was placed at the center of the hindlimb (HL) receptive field, mapped by IOS in advance. The brain activity to HL stimulation was collected in the different depth of the somatosensory cortex. **B)** Amplitude of cortical response was small on the surface and larger as electrode was moved inside the cortex. Between depth 300 μm and 400 μm , a large response was observed. By moving the recording electrode further down into the cortex (passed 400 μm), the response started to get smaller. It almost vanished at 800 μm below the surface. The red arrow shows the stimulation artifact, and maximum response is seen around 20msec after stimulation onset.

3.3.3.3 Local Field Potential (LFP) Recording and Data Analysis

During the recording sessions, animals were anesthetized under 1% isoflurane:air. Body temperature was monitored and maintained at 36.5° C by using a rectal probe and feedback-controlled heating pad. The recording electrode was inserted to 350 μ m below the surface through the center of the HL receptive field. A drop of mineral oil was applied to the exposed brain area to keep it moist. 30 minutes after the electrode insertion, SER recording was started. HL was stimulated at 100Hz 7 times for each trial (Figure. 25A). LFPs were obtained with 1700 Differential AC Amplifier (A-M system), amplified ($\times 1000$), filtered (lowpass: 0.1 Hz, high pass: 1KHz), and digitized (20KHz) using an Instrutech18™ controlled by HEKA ChartMaster™. Data were transferred to IgorPro™, and response amplitudes measured using the NeuroMatic package.

To measure the LFP amplitude, the baseline value (average of 30ms of brain activity prior to stimulation onset) was subtracted from the average value of 1ms on each side of the greatest negative deflection occurring within 20ms after the stimulation artifact (maximum peak response) (Figure.21). Mean LFP was averaged across stimulation trains (20 trials) for each condition, then across mice for each group (RTT and WT).

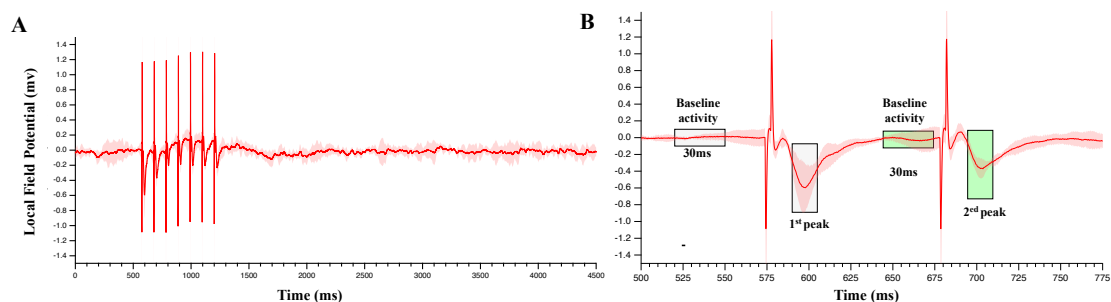


Figure 21, Measuring the peak of amplitude in the Field Local Potential recording

A) An average wave of 20 trials was averaged. B) The LFP amplitude was measured by subtracting the pre-stimulus activity from the greatest negative deflection occurring within 20 ms after the stimulation artifact for each peak.

3.3.4 Peripheral Nerve Recording

To study whether the primary afferent response was altered by sustained HL vibration compound sensory nerve action potentials (SNAPs) from the sciatic nerve were recorded while the same sensory adaptation protocol was applied.

3.3.4.1 Surgery

During the surgery, mice were anesthetized with 1.5% isoflurane:air. . Each mouse was head-fixed in an upright posture on a recording stage. Body temperature was monitored with a rectal probe and maintained at 36.5 °C with a heating pad. The depth of anesthesia was checked by the response to a toe pinch and respiration depth and rate monitored visually.

Hair on the right-side lower back of the mice, between the spinal cord and back paw was shaved and the skin cleaned with 70% ethanol. A lateral skin incision was made between the knee and spinal cord. The skin was pulled back from the center cut outwards to expose muscles. To reveal the sciatic nerve, the gluteal muscle was separated from the biceps femoris muscle. Using fine-tipped forceps, the sciatic nerve was gently freed from the surrounding connective tissue with care to avoid stretching or damaging the nerve. The sciatic nerve was cut close to the spinal cord (1.5 cm away from the paw). The distal 2mm of the nerve was suctioned inside a plastic recording pipette filled with brain buffer. The tip of the pipette was sized to hold the nerve tightly. A silver wire was located on a muscle close to the sciatic nerve served as the reference electrode. A third wire, located under the skin close to the tail, provided a system ground (Figure. 22). To avoid drying of the tissue at the incision area, a 50:50 mix of Vaseline™ and mineral oil was applied.

30 minutes after the nerve was inserted into the Eppendorf pipette, the HL stimulation was started. HL was stimulated at 100Hz, seven times for each trial, and five trials were recorded for each time point (to induce adaptation, the same protocol was applied).

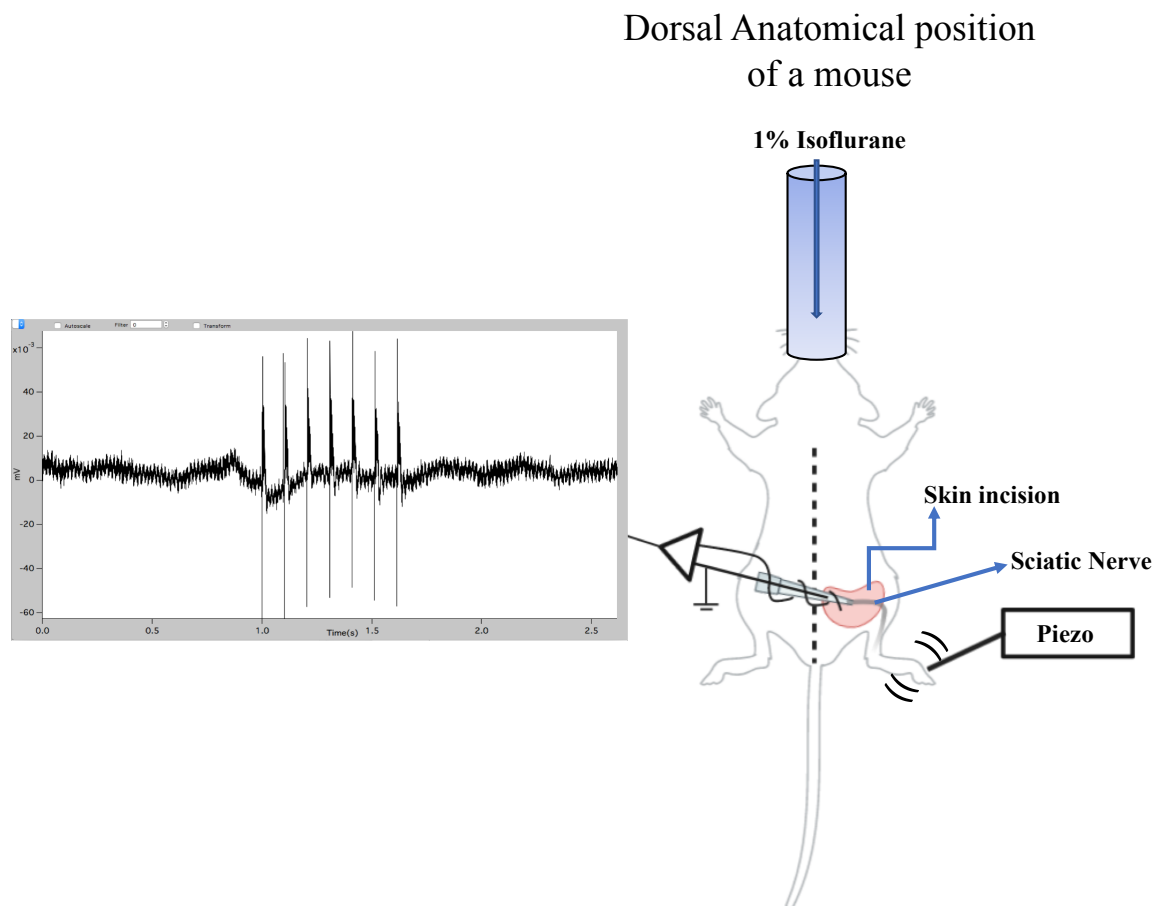


Figure 22, Schematic picture of in vivo sciatic nerve recording.

Animals were anesthetized under 1.5% and 1% isoflurane during the surgery and nerve recording sessions, respectively. A small skin incision was made on the right side of the animals to expose the sciatic nerve between the knee and spinal cord. The sciatic nerve was cut close to the spinal cord, suctioned inside a pipette filled with brain buffer, and held a recording wire. During recording the Compound Sensory Nerve Action Potentials (SNAPs), hindlimb was stimulated seven times with an electric piezo at a frequency of 100Hz, and an average of 5 trials was collected.

3.3.4.2 Data analysis:

To measure the SNAPs, an average of five trials was obtained. The area under the curve was calculated and this area was limited to the nerve impulses following the stimulation artifact for a duration of 13 ms. The area under the curve was measured and not the peak amplitude because the area is a better reflectance of the numbers of contributing axons (Rich & Brown, 2018). To eliminate the baseline activity, an area under the curve before stimulation onset (for 13 ms long) was deducted from the area under the curve of nerve response to stimulation. Mean value $\pm$ SE was calculated and plotted.

3.4 Results

3.4.1 Cortical plasticity, persistent sensory adaptation, following a repetitive hindlimb stimulation is impaired in male *Mecp2* null mice

We established a baseline sensory-evoked response (SER) Intrinsic Optical Signal (IOS) response in hindlimb (HL) somatosensory cortex of *Mecp2* mutant (RTT) and age-matched wild-type (WT) controls to 100 Hz, 1 second (HL) vibration, averaging five responses delivered at an interval of 14 seconds in two sessions separated by 15 minutes. To induce persistent sensory adaptation (PA), the HL was stimulated with the same 100 Hz, 1-second stimulus delivered with an inter-stimulus interval of 6 seconds for 15 minutes. The average SER was measured following a 2 minutes rest period, and the continuous stimulation resumed for three additional 15 minutes stimulation/test periods. Continuous stimulation was then stopped, and the SER was monitored at 15 minutes intervals for an hour.

In WT (n=6), the optically assessed SER declined to $46 \pm 2\%$ of its pre-stimulus level (for 60 minutes of sustained HL vibration). The SER did not return to its pre-stimulus baseline response level for at least 60 minutes of recovery ($p < 0.0001$). Drop in the SER value occurred gradually; however, the last 15 minutes of stimulation did not further suppress the SER (Figure. 23A). This result indicates that 45 minutes of continuous sensory stimulation in the WT group was enough to induce the maximum adaptation in this sensory pathway. After cessation of the repeating vibratory stimulus, the SER recovered slowly, reaching $73.6 \pm 2.8\%$ of baseline SER ($p = 0.0015$) at 45 minutes and $79.3 \pm 3.9\%$ ($p = 0.007$), at 1 hour when the recording was terminated. In the non-stimulated sham group, there was no change in the magnitude of SER during the 2-hour testing period (Figure. 23E).

In RTT animals (n=6), SER was reduced by $37.2 \pm 1.5\%$ during the adaptation phase, but in contrast to WT animals, it recovered quickly. During the adaptation, brain SER was

decreased by $29.45 \pm 1.09\%$ after 15 minutes of HL stimulation and with an additional 15 minutes of stimulation declined a further $6.98 \pm 0.47\%$. Continuing HL stimulation did not reduce the magnitude of SER further (Figure. 23B). In clear contrast to the recovery in WT mice, the SER increased rapidly after rest, reaching $83.45 \pm 2.2\%$ of initial response ($p=0.0096$) after 15 minutes and $92 \pm 3.6\%$ of the baseline SER after 45 minutes ($p=0.012$). SER of the sham group was maintained during a 2-hour testing period (Figure. 23E).

Comparing the magnitudes of SER in WT to RTT during the adaptation and recovery phases showed that the RTT started with a higher SER, which remained higher throughout the experiment ($p<0.0001$) (Figure. 23C). Normalizing data to the baseline value indicated that the adaptation was proportionately similar in RTT and age-matched WT; however, there is a significant difference in the recovery phase ($p<0.0001$). Comparing RTT to WT, the fractional ΔR was greater in RTT measured at 15 minutes ($p=0.0001$), 30 minutes ($p=0.0001$), and 45 minutes ($p=0.0004$) after the termination of the continuous vibratory stimulus (Figure. 23D).

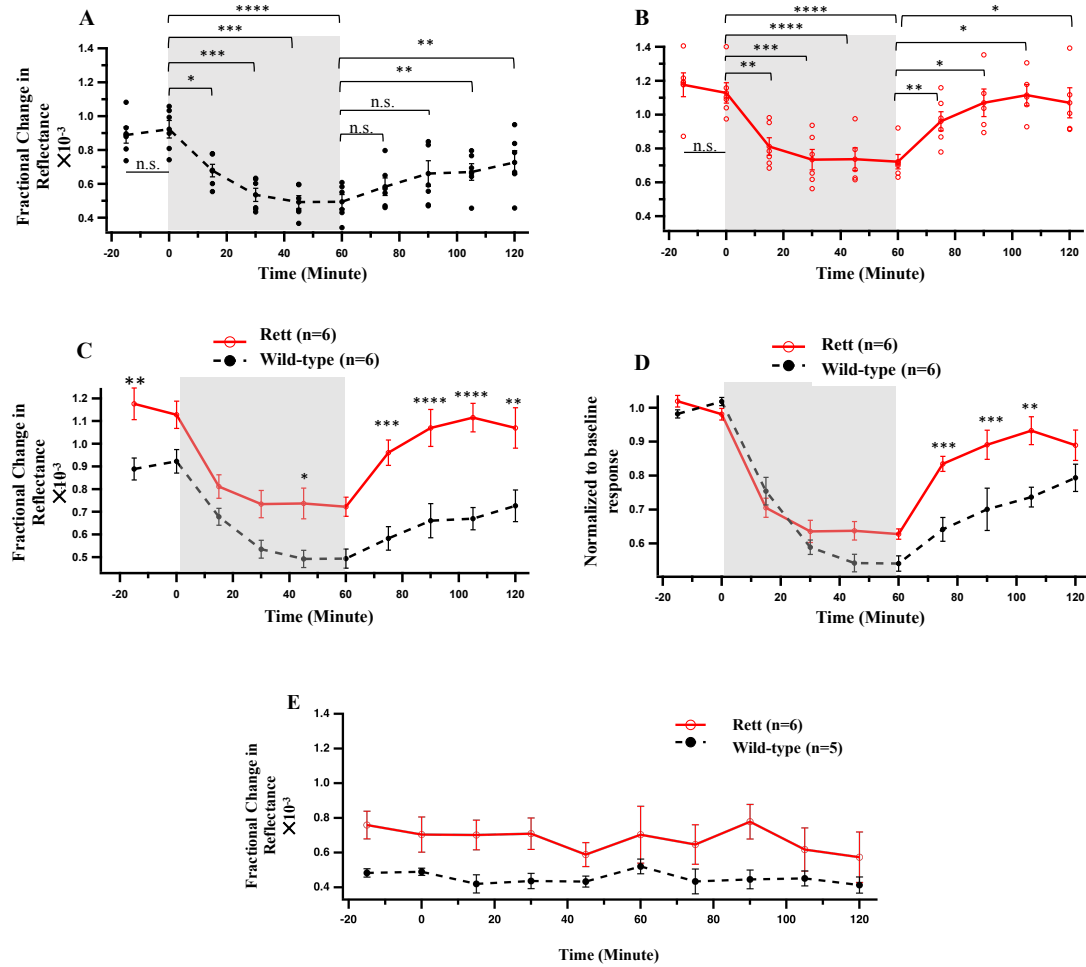


Figure 23, Fractional change in reflectance of cortical evoked-response due to repeated hindlimb stimulation (100Hz, 1sec, ISI 6sec)

A) Intrinsic Optical Signaling (IOS) imaging of 4-6-week-old male wild-type (WT) mice (n=6) showed cortical SER was reduced by repeated hindlimb (HL) stimulation (grey region) ($p < 0.0001$). **B)** SER of 4-6-week-old male Rett (RTT) mice (n=6) ($p < 0.0001$). **C)** Initial cortical SER (baseline) is higher in RTT and remained greater throughout the experiment duration ($p < 0.0001$). **D)** SER normalized to baseline SER. **E)** Sham groups lacking repeated stimulation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

As discussed in Chapter 2, IOS imaging reflects changes in blood oxygen level (BOL) (Frostig & Chen-Bee, 2009). Symptomatic Rett animals often have breathing impairments (Ogier & Katz, 2008). Therefore, we monitored the BOL of WT and RTT during the experiment. Although there is more variation in the BOL between individual mice in the RTT group, it was constant for the 2 hours experimental period ($p=0.60$) (Figure. 24). This suggesting the observed changes in ΔR magnitude during the experiment were not dependent upon changes in BOL.

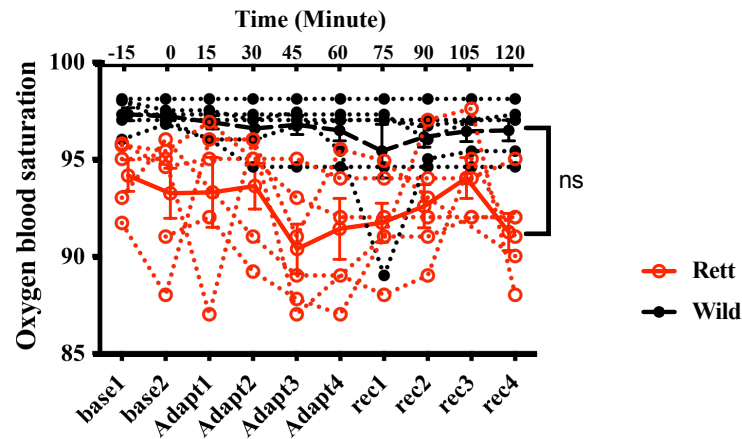


Figure 24, Blood oxygen saturation level in 4-6-week-old male mice over 2 hours of Intrinsic Optical Signaling (IOS) Imaging is stable

Blood oxygen saturation was constant across 2 hours in RTT and WT groups ($p=0.60$).

Additionally, comparing the magnitude of baseline SER in RTT and WT, both sham and experimental groups suggested a significantly greater cortical SER to the same sensory stimulation in the RTT group (Figure. 25A). To investigate whether the difference in the cortical SER between these two groups is a result of the lower BOL in RTT (Figure. 25B), the correlation between BOL and ΔR was calculated (Figure. 25C). Although there is a high variability of the BOL in the RTT group, there is no correlation between these two parameters ($p=0.6$), meaning the change in the BOL does not affect the SER magnitude in RTT nor WT ($p=0.49$).

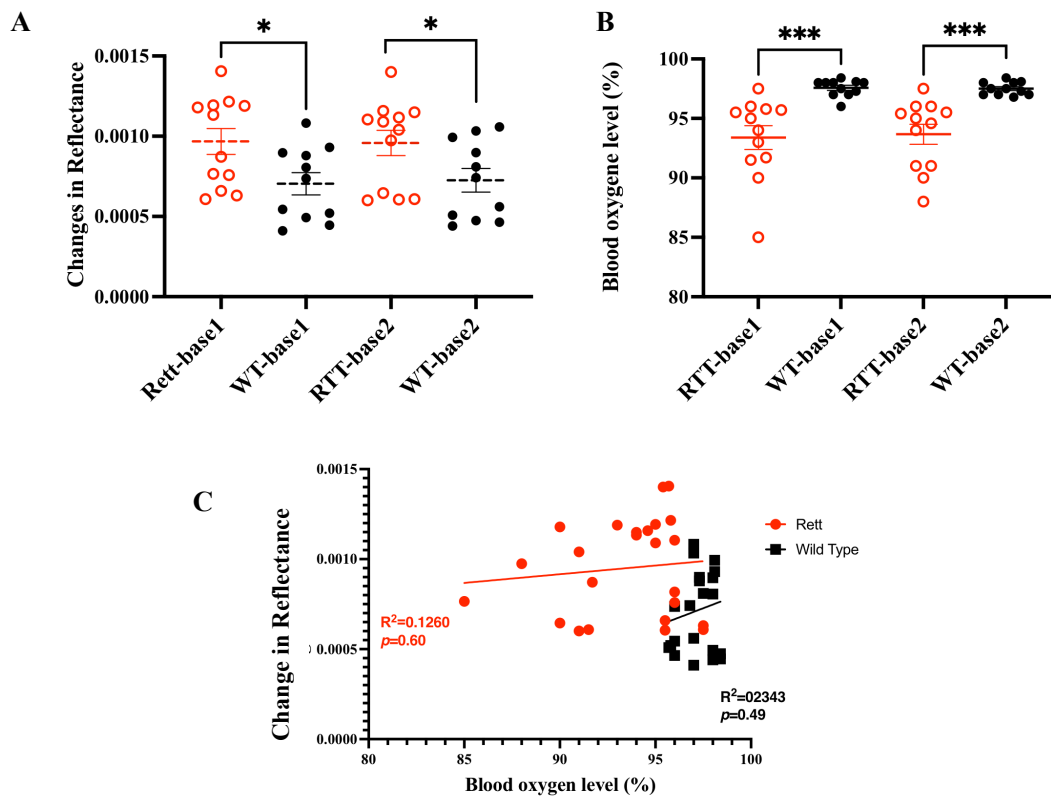


Figure 25, Possible hypersensitivity in 4-6 weeks old male Rett model mice to vibration stimuli

Data points include both sham and test animals. **A)** The SER magnitude was larger in RTT mice. **B)** The Blood oxygen level is lower in RTT mice **C)** There is no correlation between blood oxygen level and the magnitude of change in reflectance (ΔR) in either group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

These results suggest a possible hypersensitivity to vibratory stimuli in *Mecp2*^{-y} animals, similar to the collected data from the whisker stimulation in the previous chapter. Hypersensitivity to a gentle touch in Autism Spectrum Disorder (ASD) and RTT model mice has been reported, ascribed to reduced presynaptic inhibition in the spinal cord (Orefice et al., 2016).

3.4.2 Persistent sensory adaptation following a repetitive hind limb stimulation is impaired in pre-symptomatic and symptomatic *Mecp2*^{+/-} female mice

Since the *de novo* mutations producing rate occur primarily on the paternally derived X chromosome (Trappe et al., 2001), there is a high female:male ratio among RTT patients. To investigate PA in symptomatic female Rett (RTT-F) model mice, the SER to HL stimulation was measured with IOS imaging in 2-month and 10-12-month-old females.

Our results for 10-12 month highly symptomatic mice are presented first, followed by data for pre-symptomatic 2-month-old mice. 10-12-month RTT-F are reliably heavier than age-matched wild-type females (WT-F), 46.02 ± 2.6 compared to 29.02 ± 1.48 gram. ($p < 0.0001$) due to the accumulation of fatty tissue (Figure. 26A). RTT-Fs also show other overt symptoms, including HL clasping (Figure. 26B), wobbly locomotion, and intermittent periods of seizure activity revealed as bouts of immobility and mild shaking.

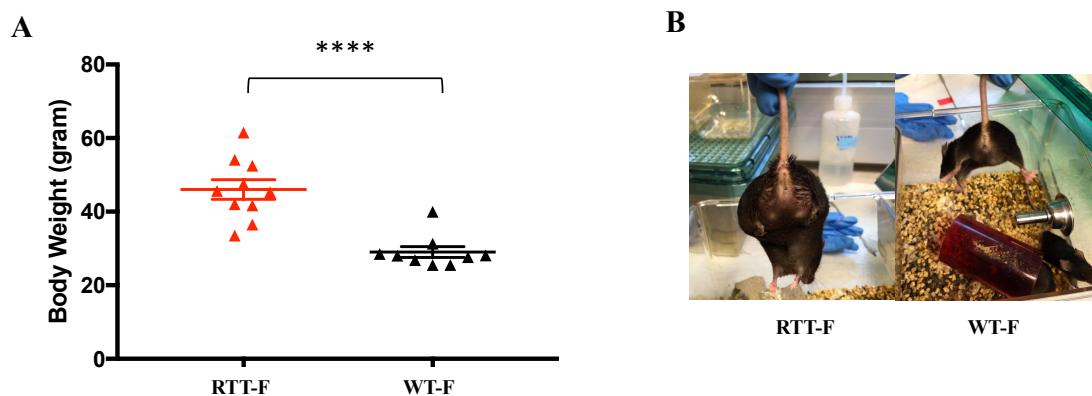


Figure 26, One-year-old *Mecp2*^{+/-} mice show symptoms including high body weight and hindlimb clasping

A) Rett old females (RTT-F) were significantly heavier than age-matched wild-type (WT-F) ($p < 0.0001$). **B)** RTT mice exhibited hindlimb clasping.

During one hour of HL stimulation (100Hz, 1 sec, 6 sec ISI), the SER was equally reduced to $49.5 \pm 6\%$ and $51.8\% \pm 5\%$ of pre-adaptation levels in RTT-F and WT-F respectively ($p < 0.0001$) (Figure 27A & B). For both RTT and WT, the majority of the recovery of SER occurred within 15 minutes. However, for WT, the recovery was only moderate, to about $71.11 \pm 6.7\%$ of pre-adaptation levels and remained significantly reduced at about this level throughout the next 45 minutes. In sharp contrast, RTT-F SER recovered to about 90% of pre-adaptation levels after 15 minutes of rest and reached pre-adaptation levels by 30 minutes (Figure. 27). SERs were unchanged in the sham groups, confirming the stability of SER during the experiment duration (Figure. 27D).

We monitored the BOL and found it similar for both groups (96%) during adaptation and recovery phases (Figure. 27E). This suggests that the difference in ΔR -value between the two groups during the recovery phase was independent of the basal BOL.

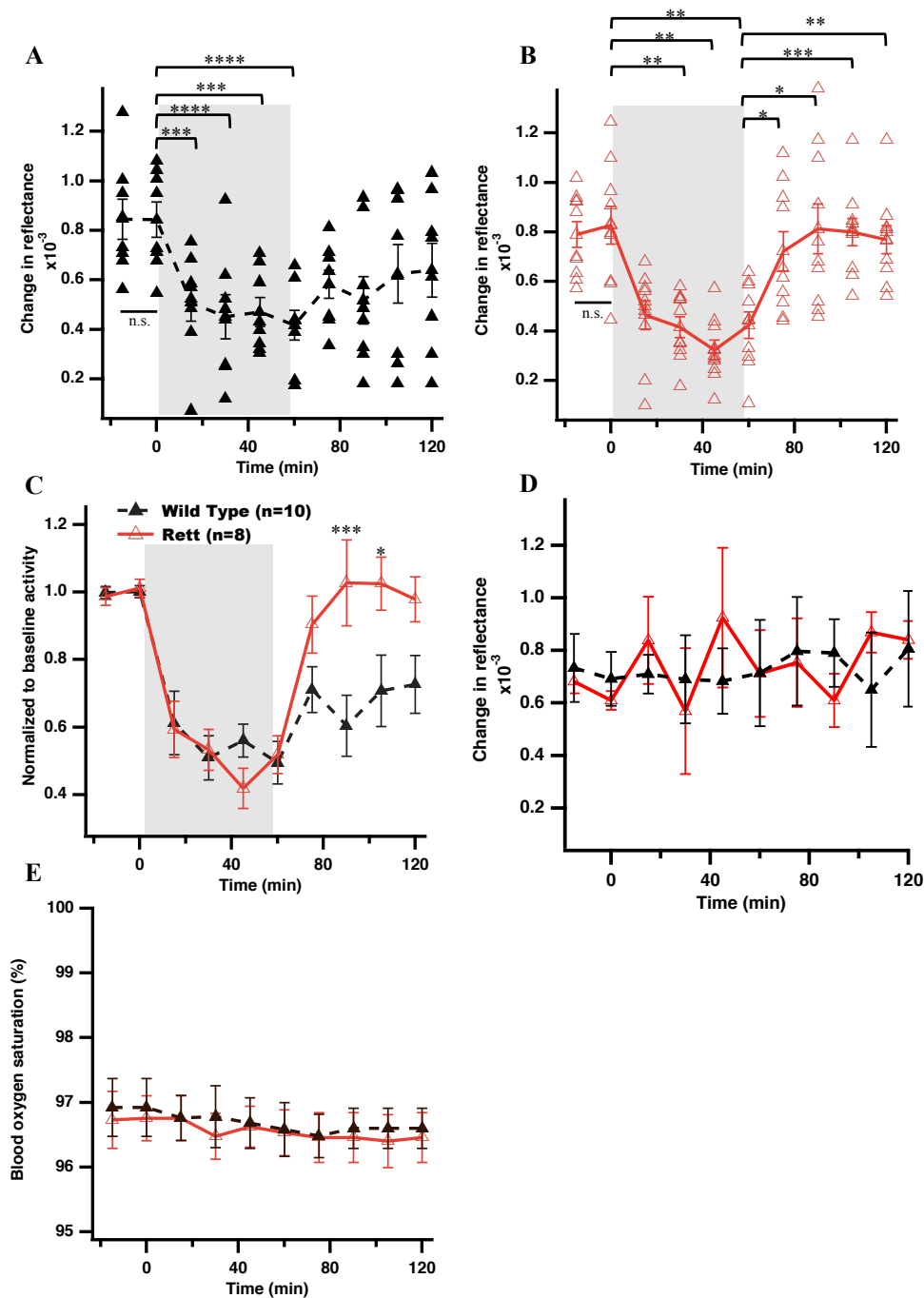


Figure 27, Equal adaptation during 1 hour of repeated hindlimb (HL) stimulation and rapid reversal in RTT-F (10-12-month-old females)

A) 10-12-month-old wild-type female (WT-F) mice (n=8) showed reduction in the cortical sensory evoked response (SER) as a result of repeated HL stimulation (100Hz, grey shaded region) with little evidence for recovery of response up to 60 minutes later ($p < 0.0001$). **B)** Age-matched heterozygous females (RTT-F) mice (n=10) adapted during repeated HL stimulation, but the adaptation did not persist and responses recovered substantially after 15 min and reached pre-adaptation levels after 30 min ($p < 0.0001$). **C)** Normalized value to baseline SER ($p = 0.0002$). **D)** Sham groups. **E)** Blood oxygen saturation during 2 hours of the experiment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

To investigate whether the fast recovery following the adaptation phase in the symptomatic Rett model mice results from symptom development or might be one of the causes of future syndrome severe impairments in symptomatic patients, the adaptation protocol was applied to pre-symptomatic Rett female model mice (RTT-YF). Using 2-month-old female mice addressed both the questions of whether differences between RTT and WT reflected an age effect.

2-month-old female Rett model mice (RTT-YF) do not show any symptoms, and body weight is identical to age-matched wild-type ($p=0.26$) (Figure. 28).

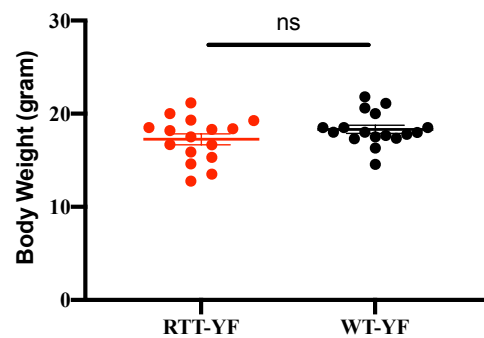


Figure 28, 2-month-old *Mecp2*^{+/-} and age-matched wild-type are the same weight

Body fat mass in heterozygous female mice (RTT-YF) is similar to age-matched wild-type (WT-YF) ($p=0.26$).

IOS imaging showed that in WT-YF ($n=9$), the magnitude of cortical SER dropped by $35.17 \pm 5.4\%$ in the adaptation phase and recovery was not seen for at least one hour ($p=0.0001$). Drop-in SER magnitude started to be significant after 30 minutes of HL stimulation ($p=0.007$), in which the SER magnitude was $78 \pm 3.5\%$ of pre-stimulus baseline. The cortical SER did not change in the last 15 min of HL stimulation, suggesting 45 minutes of HL stimulation was enough to induce the maximum adaptation in WT-YF (Figure. 29A), similar to the 2-month-old WT male mice. There was no change in the magnitude of the non-stimulated sham group over the 2-hour experiment (Figure. 29D).

In the RTT-YF group (n=8), SER magnitude declined by $43.38 \pm 6.8\%$ after one hour of HL stimulation. However, in contrast to WT-YF, it returned quickly to the baseline magnitude at the recovery phase ($p<0.0001$). 30 minutes of HL stimulation caused the cortical SER reduced by $27.30 \pm 2.7\%$ ($p=0.007$). There was an additional 16% reduction in the response magnitude due to extra 30 minutes of HL stimulation. As with RTT males and old females, recovery from adaptation was rapid. 15 minutes after cessation of the continuous vibratory stimulus, SER returned to $83 \pm 5.7\%$ of the pre-stimulus value. SER continued to recover, achieving almost to its pre-stimulus magnitude after 60 minutes ($p=0.010$) (Figure. 29B). SER magnitude of the sham groups was unchanged during the experiment (Figure. 29D).

Normalized data to pre-stimulus value (baseline) showed the relative similarity in the reduction of cortical SER during the adaptation and larger recovery in RTT-YF ($p=0.0002$) (Figure. 29C).

The BOL was similar ($\sim 97.6\%$). It did not change during the imaging session for either group (Figure. 29E), meaning the larger SER at 45 and 60 minutes of the recovery phase in RTT-YF is independent of BOL.

In 2-month-old mutant females (*Mecp2*^{+/-}), the magnitude of SER in the baseline is slightly larger than WT-YF, but it is not as pronounced as the effect in *Mecp2*^{-y}. There is a mixture of MECP2⁺ and MECP2⁻ cells in the mutant female brains. Due to skewed X-inactivation, the ratio might differ in individuals, resulting in a higher variation in the female group.

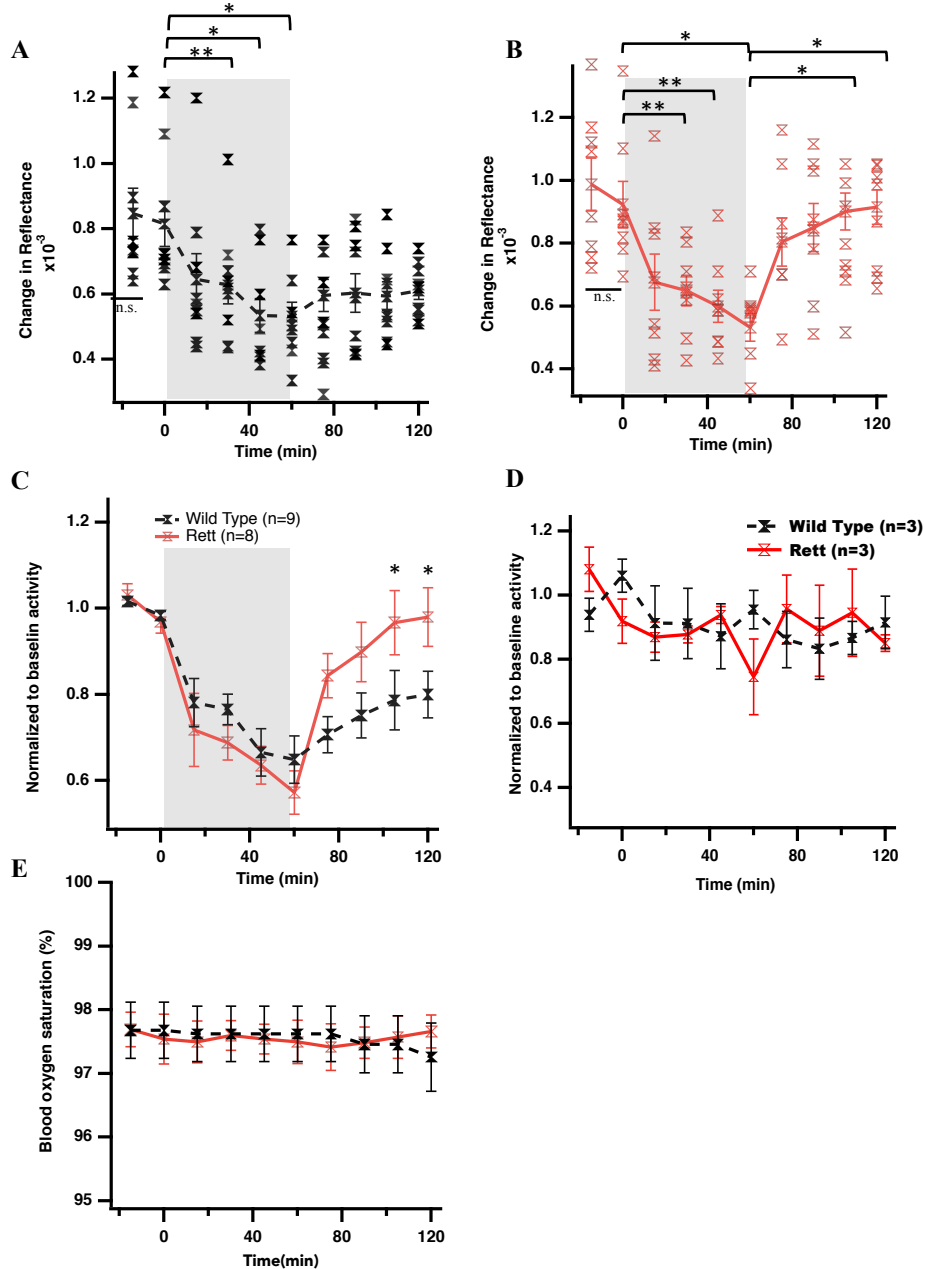


Figure 29, Intrinsic Optical Signalling (IOS) imaging of 2-month-old female Rett model mice and age-matched wild-type during adaptation and recovery phases.

A) Fractional change in reflectance (ΔR) as an indicator of cortical SER was declined in 2-month-old wild-type female mice (WT-YF) (n=9) as a result of repeated hindlimb (HL) stimulation (100Hz, grey shaded region) with the little recovery of response up to 60 minutes later ($p=0.0001$). **B)** In Rett female model mice (RTT-YF) (n=8), a 40% reduction in cortical SER was observed in the adaptation phase, which was not persistent and returned to almost the baseline level at the end of the recovery period ($p<0.0001$). **C)** Normalized SER magnitude to the baseline ($p=0.0002$). **D)** sham groups. **E)** The blood oxygen saturation during 2 hours of the experiment. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

3.4.3 Activation of feedback network is essential for cortical plasticity formation

To validate whether the change in ΔR magnitude observed during the adaptation experiment was generated by neuronal activity rather than a neurovascular coupling system, the neuronal response was recorded directly by the intracortical Local Field Potential (LFP) technique. LFP gives more insight into the mechanism of the observed PA.

To better understand the underlying mechanisms in the observed sensory adaptation, we decided to stimulate the HL more than once in each trial while measuring the SER by intracortical LFP recording. Initially, a pair pulse stimulation was applied. Neither of the peaks (1st nor 2^{ed} peak) showed any significant reduction in the amplitude during the adaptation phase (Figure. 30A). Since the observed PA with IOS imaging results from recording the cortical SER for one second, it was suggested that the sensory PA might be due to the accumulation of synaptic adaptation in the sensory pathway.

To test the hypothesis, the number of stimulations was set higher (seven stimuli, 100ms interval) for each trial during LFP recording (Figure. 30B). Therefore, the brain SER was recorded for 700ms. It was not possible to apply more pulses as the response size was getting smaller after each stimulation, and measuring the peak amplitude and calculating the amount of reduction would not be very accurate. The amplitude of peaks one (P1), two (P2), and three (P3) did not change during the adaptation or recovery phases (Figure 30C, D, E). A decline in peak amplitude began to appear at peak four (P4) (Figure. 30F), suggesting that the PA results from increasing the short-term synaptic adaptation (STA) in the sensory circuit and activation of feedback networks.

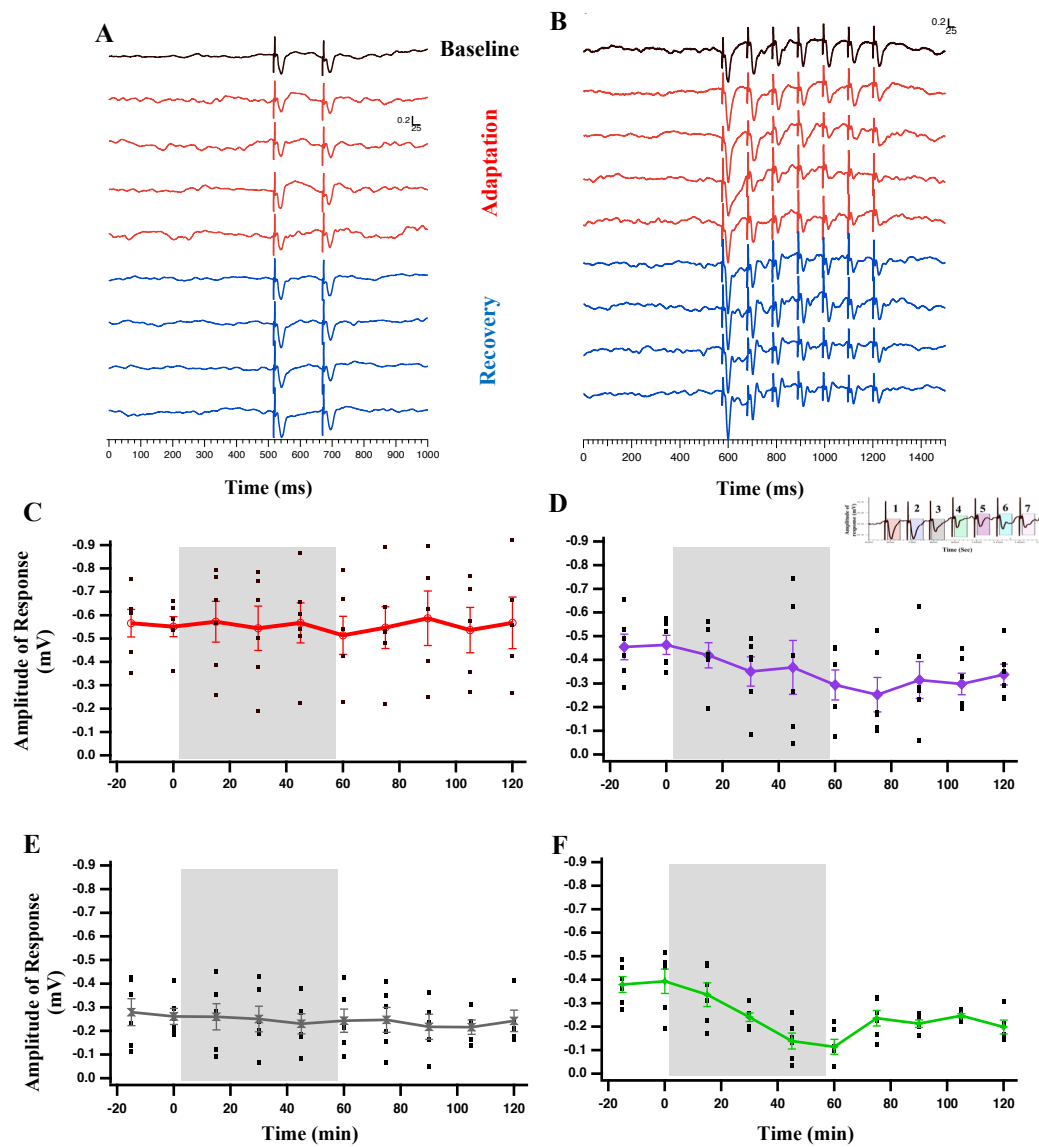


Figure 30, Persistent adaptation (PA) results from increasing the short-term adaptation (STA) in the sensory pathway from HL to S1.

A) An example of paired-pulse recording. B) An example of seven pulses recording. Peak1 (C), peak2 (D), peak 3(E), and peak4 (F) amplitude during adaptation and recovery phases.

3.4.4 Intracortical Local Field Potential (LFP) recording confirmed the sensory adaptation result collected by IOS imaging

To study the sensory adaptation with LFP recording, the amplitude responses of all peaks were calculated and summed up. This value is an indicator of brain sensory-evoked response (SER) over 700ms.

4-6-week old male

Looking at the individual peaks indicates, in WT (n=6), P1 to P3 did not show any drop at the adaptation phase, while the decline in response was observed in subsequent peaks (P4 to P7) (Figure. 31A). In RTT (n=10), a decrease in response amplitude began to appear by P2. P1 in RTT, similar to WT, shows no change during the adaptation phase, but in contrast to WT, there is an overshoot in recovery time (Figure. 31B).

In WT, the baseline activity was constant at 15-minute intervals ($p=0.74$). The SER reduced to $57 \pm 8.9\%$ of initial response in the adaptation phase and recovery was not seen in this group ($p=0.0004$) (Figure. 31A&C). SER reduced gradually: the first 15 minutes of continuous HL stimulation did not change the size of the evoked response. 30 minutes of stimulation led to $26 \pm 6.4\%$ dropped in SER amplitude ($p=0.0143$). An additional 12.41% decline was observed at 45 minutes of the adaptation phase ($p=0.0075$). After 60 minutes of recovery, the SER amplitude did not rise back, similar to the IOS imaging result.

RTT animals exhibited around a 47% reduction of cortical SER amplitude at the adaptation phase and quick recovery ($p<0.0001$) (Figure. 31B&D). Like WT, RTT had a stable baseline SER for 15 minutes ($p=0.37$). Adaptation took place progressively; a 15-minute HL stimulation caused a $20 \pm 4.8\%$ drop in the cortical SER ($p=0.0195$). HL stimulation for an additional 15 minutes resulted in a $13 \pm 3.1\%$ further decrease ($p=0.0178$). The SER was down

to $53 \pm 5.1\%$ of the initial level after 45 minutes of HL stimulation ($p= 0.0015$), and finally, by one hour of HL stimulation, it was $52.8 \pm 4.1\%$ of baseline level ($p= 0.0005$). In contrast to the gradual reduction during the adaptation phase, the recovery occurred quickly. 15 minutes of recovery time led to a $44.6 \pm 6.7\%$ increase of SER ($p= 0.026$). After 30 minutes, the response amplitude was almost as large as the pre-stimulus level.

Comparing the trend of SER amplitude in RTT and age-matched WT during the adaptation phase indicated the degree of decline in the magnitude of SER in both groups is indistinguishable. At the same time, the recovery was fast in RTT ($p < 0.0001$), which is significant at 30 minutes ($p=0.0142$) and 45 minutes ($p= 0.0004$) of recovery time (Figure. 31E).

In sham groups, the amplitude of individual peaks (P1 to P7) was constant in RTT ($n=5$) ($p=0.8061$) and age-matched WT ($n=4$) ($p= 0.3701$) for two hours, indicating that anesthesia does not affect the SER magnitude during the experiment (Figure. 31E).

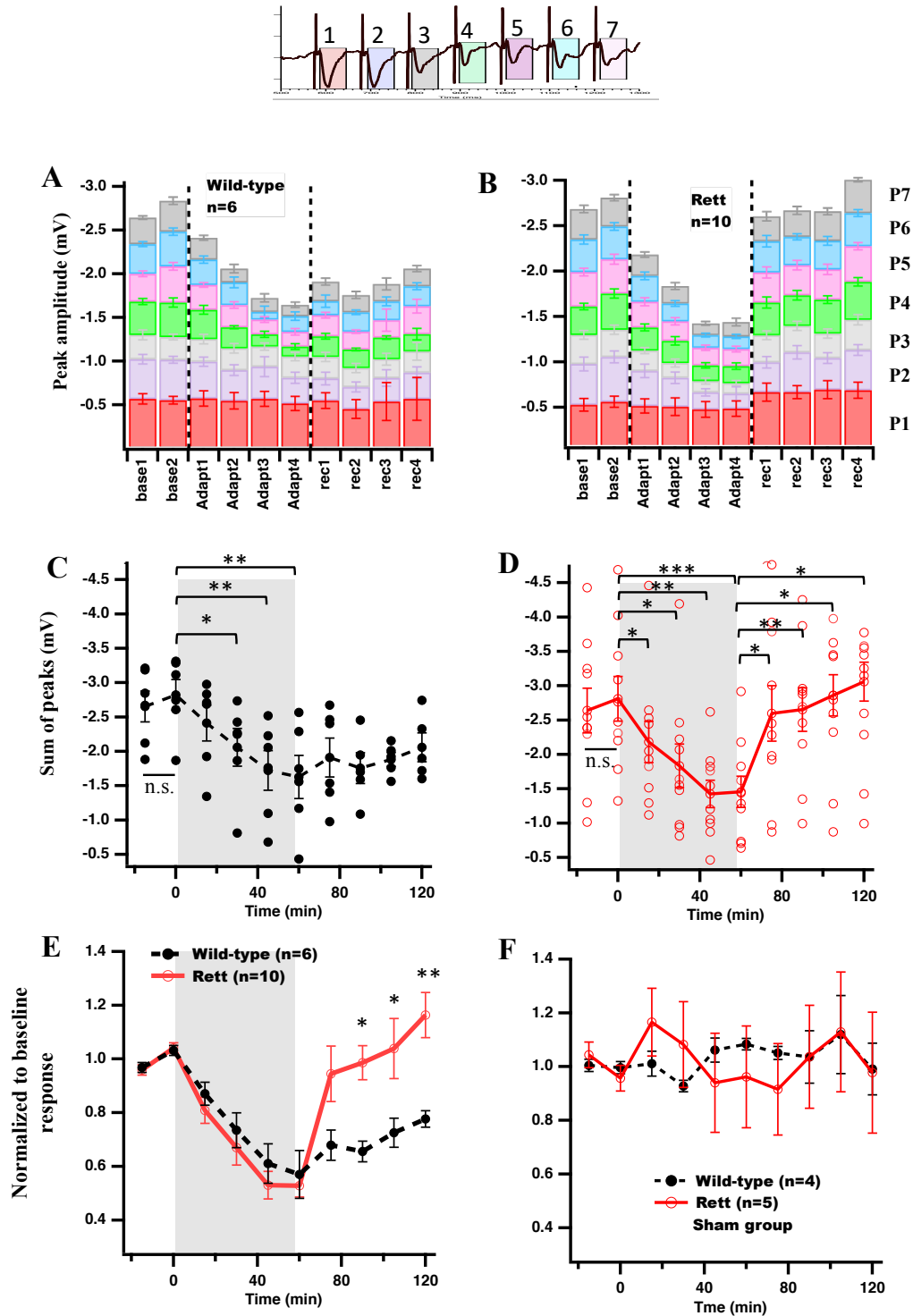


Figure 31, Intracortical LFP recording of 4-6-week-old male mice (350 μ m below the surface) during adaptation and recovery phases.

A&B) Change in the amplitude of individual peaks (P1 to P7) during adaptation (dotted lines) and recovery in wild-type (WT) mice ($n=6$) and Rett mice (RTT) ($n=10$), respectively. **C& D)** Change in the sum of the seven peaks during the adaptation (gray square) and recovery phases in WT ($p=0.0004$) and RTT ($p<0.0001$). **E)** The same rate of decline in the adaptation phase for WT and RTT mice and fast recovery only in RTT ($p<0.0001$). **F)** sham groups ($p=0.8604$). * $p<0.05$, ** $p<0.01$, *** $p<0.001$

10-12-month-old female

Opposite of younger groups, in older mice, P1 amplitude was reduced and increase during the adaptation and recovery phases, respectively (Figure. 32 A&B).

In the WT-F mice model (n=5), the SER dropped by almost 40% in the adaptation phase, and there was no recovery at least for 60 minutes ($p=0.001$) (Figure. 32 C). The sum of the amplitude of all seven peaks (Figure.32C) showed the drop in cortical SER amplitude during the adaptation phase occurred in 2 steps; 15 minutes of HL stimulation reduced the SER by 22%, and from there to adapt3 (45 min of HL stimulation) the magnitude of response remained stable. An additional 15 minutes of HL stimulation (total of 1-hour stimulation) caused an 18.8% further decline in SER.

In symptomatic RTT-F (n=8), the SER magnitude decreased by 42% in the adaptation phase, and it went back up to baseline level 15-30 minutes after the stimulation cessation ($p<0.0001$) (Figure. 32 D). Reduction of SER magnitude started right after 15 minutes of HL stimulation; it dropped about 43% ($p=0.0007$). The SER magnitude did not change due to further HL stimulation (no significant change in the amplitude of response at adapt1-4), meaning that in the RTT-F group, sensory adaptation occurred very quick. Recovery was also rapid in this group. 15 minutes of recovery time resulted in a rising SER to almost the baseline magnitude ($p=0.0092$).

Normalizing the SER magnitude of each stage to the baseline indicated no significant difference in the adaptation phase between RTT-F and age-matched WT-F. In contrast, the recovery was seen over 15 minutes in RTT-F and not in WT-F ($p=0.0002$) (Figure. 32E). The SER amplitude was stable for two hours in the sham groups (Figure. 32F).

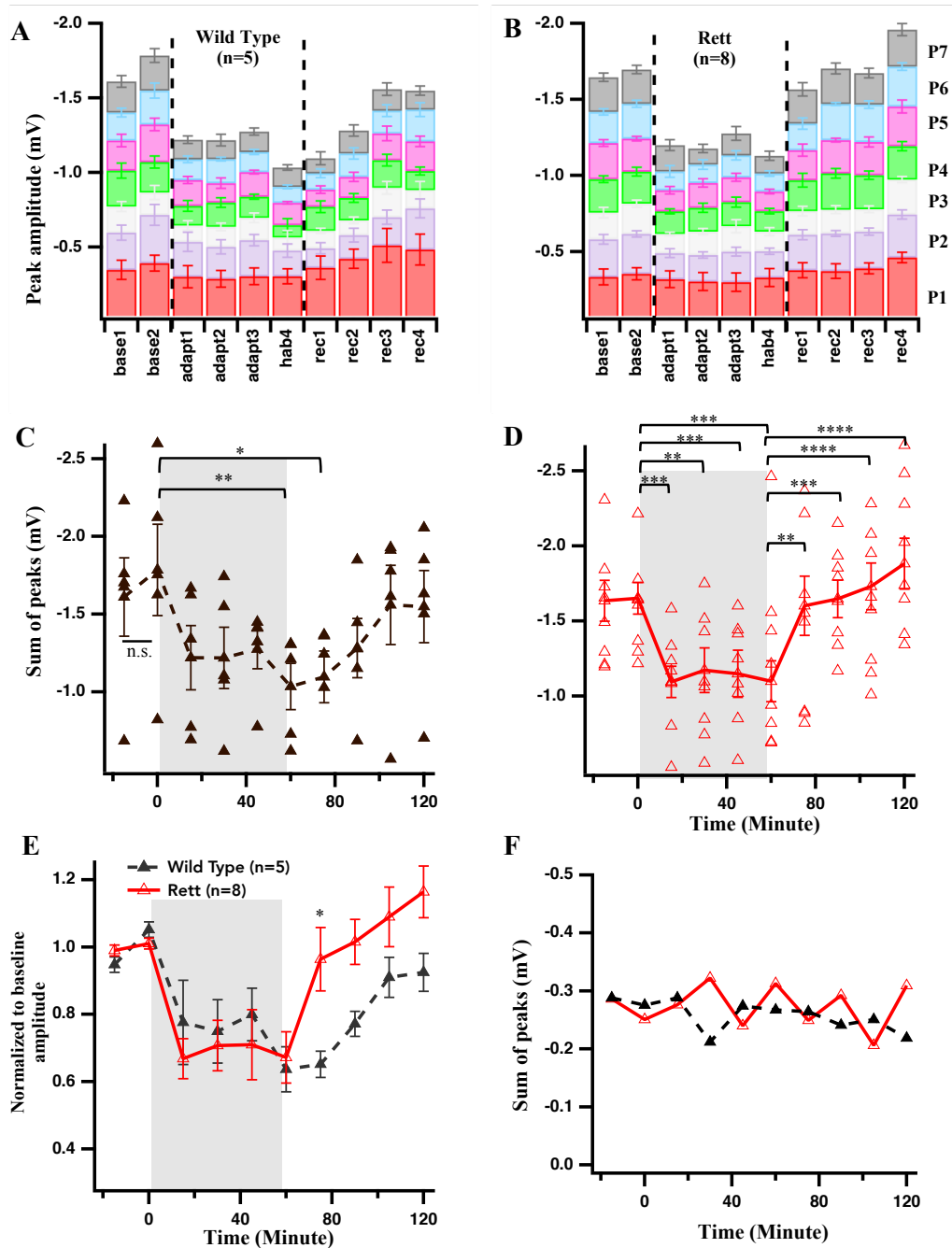


Figure 32, Local Field Potential recording of 10-12-month-old Rett (RTT-F) and wild-type (WT-F) female mouse showed a reduction of SER as a result of repetitive hindlimb (HL) stimulation in both groups and a quick recovery only in RTT-F.

A & B) The change in the amplitude of individual peaks during the adaptation (dotted lines) and recovery phases in WT-F (n=5) and RTT-F (n=8) groups, respectively. **C & D)** Change in the sum of the seven peaks during the adaptation (gray square) and recovery phases in WT-F ($p=0.001$) and RTT-F ($p<0.0001$) during the adaptation recovery periods, respectively. **E)** The rate of adaptation is similar between RTT-F and WT-F, while the recovery is faster in RTT ($P=0.0002$). **F)** Sham groups. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

2-month-old female

In WT-YF, similar to the 4-6-week-old WT male group, the magnitude of P1 remained almost constant during the adaptation and recovery phases. P2 and P3 showed a slight drop, and at P4, a more significant decline in peak amplitude was observed at 15 minutes of the adaptation phase (adapt1) (Figure. 33A). This is another piece of evidence that goes along with the previous results that showed the observed sensory adaptation in WT animals resulting from an accumulation of synaptic adaptation. It indicates that feedback networks in sensory systems are essential to reveal PA in the sensory pathway.

In the RTT-YF (n=5) group, the drop in the SER amplitude was noticeable at all the peaks (Figure. 33B), similar to the results collected from symptomatic RTT animals (male and older female mice). This suggested that the mutation of *Mecp2* causes instability in the sensory pathway even before symptoms have appeared.

The sum of the amplitude of all seven peaks showed about a 40% reduction in WT-YF ($p<0.0001$) and RTT-YF ($p<0.0001$). RTT-YF showed a full recovery at 15 minutes of recovery phase while it was only 20% in WT-YF even 60 minutes after stimulation cessation (Figure. 33C&D).

Normalizing the SER of individual animals in each stage (adapt1-4 and rec1-4) to the pre-stimuli SER magnitude showed the approximately similar reduction in the adaptation phase and only recovery in RTT-YF ($p<0.0001$). Multiple comparisons test indicated significant differences between rec1 ($p=0.001$), rec2 ($p=0.025$), rec3 ($p=0.0467$), and rec4 ($p=0.0472$) (Figure. 33E). There was no change in the SER magnitude in sham groups, indicating that the anesthesia did not affect the brain's sensory-evoked response over the experiment duration (Figure. 33F).

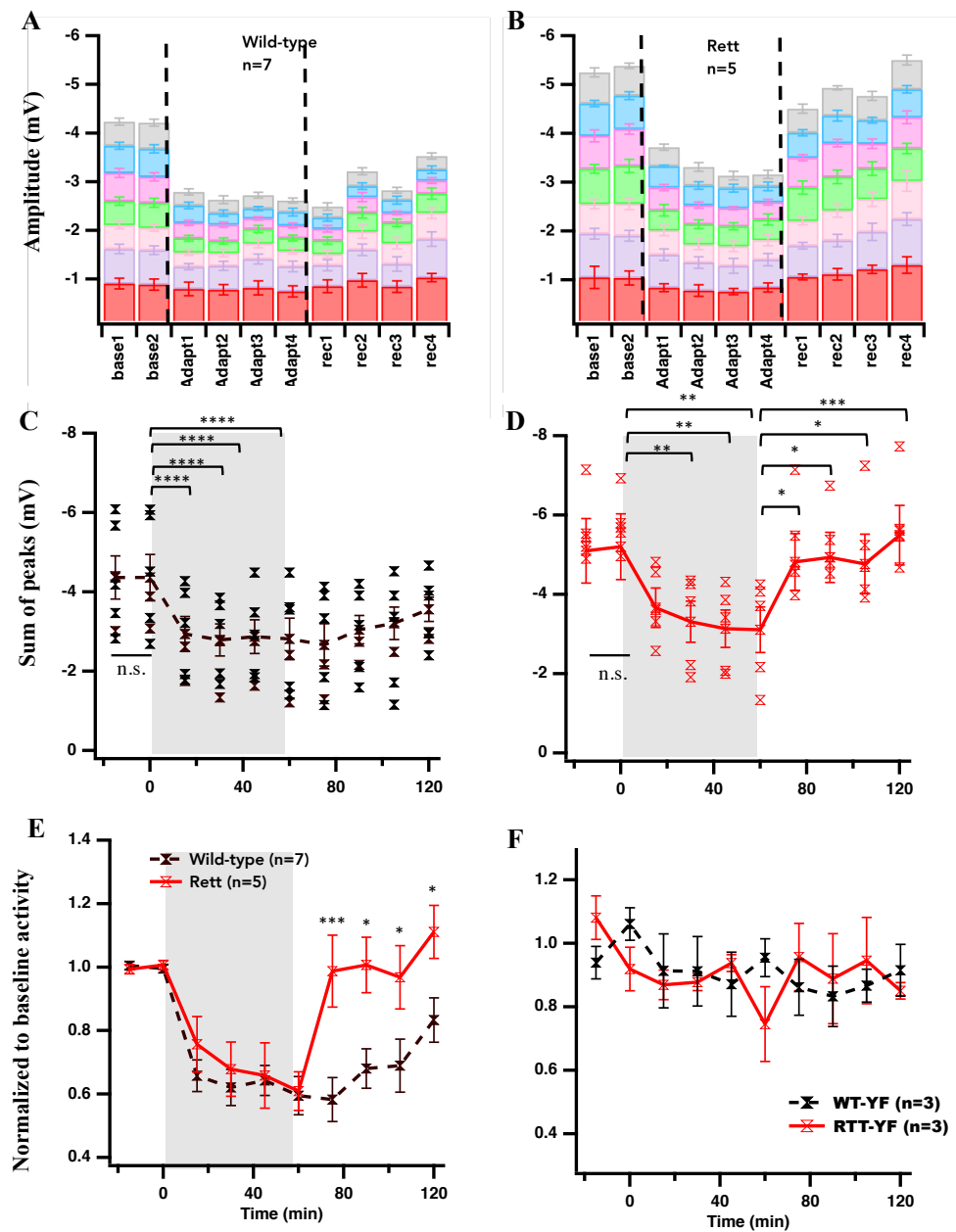


Figure 33, Intracortical Local Field Potential (LFP) recording in 2-month-old female mice

A&B) Change in the amplitude of individual peaks (P1 to P7) during adaptation (dotted lines) and recovery in wild-type (WT-YF) mice (n=7) and Rett mice (RTT-YF) (n=5), respectively. **C)** Sum of all peak's amplitude in WT-YF showed a 40% reduction in adaptation phase (gray square) and no recovery at least for 60 min ($p < 0.0001$). **D)** Sum of the peak's amplitude in RTT-YF showed a 40% drop and complete recovery ($p < 0.0001$). **E)** Normalized the SER at each stage to baseline level. **F)** sham groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Data from all the WT groups indicated the magnitude of P1s remain constant during the adaptation phase while the subsequent peaks decline. It suggests that the sensory adaptation during the 1 hour of HL stimulation could be due to increasing the level of short-term adaptation (STA) in the HL to S1 pathway. To test this hypothesis, the rate of STA was measured at pre-stimuli, 60 minutes of adaptation, and recovery phases. The following section will provide more insight into the underlying mechanism of the persistent adaptation in WT and the lack of it in RTT group.

3.4.5 Short-term adaptation is similar in *Mecp2* mutant and age-matched wild-type mice

One of the dynamic properties of neuronal synapses is the ability of a synapse to respond differently to two stimulation pulses separated by a short interval. The second response could be smaller (depression) or more prominent (facilitation) than the first response (Bouteiller et al., 2010). Here we evaluated the STA pattern due to seven stimuli 100ms inter-stimuli intervals (ISI) at the pre-adaptation phase in all experimental groups to investigate the impact of MECP2 on STA.

STA was observed in 4-6-week old male mice, WT ($p=0.0004$), and RTT ($p<0.0001$) with no change in the amplitude of P1 and P2 but a decline in P3 to P7. To investigate the degree of STR, the amplitude of each peak was normalized to the first peak amplitude. A 50% reduction in P3 was seen, in addition to a slight increase (about 10%) from P3 to P4 and a plateau from P4 to P7. This pattern was the same in both groups (Figure. 34A), meaning the STA pattern is identical in the baseline stage between these two groups.

Like male groups, 2-month-old females exhibited the STA in both RTT-YF ($p=0.0012$) and WT-YF ($p<0.0001$). There was no significant difference in the rate of STA between these groups ($p=0.7172$) (Figure. 34B).

STA in old groups, both WT-F ($p=0.0211$) and RTT-F ($p=0.0093$) showed similar pattern ($p=0.8371$) (Figure. 34C).

Together, this data indicates that *Mecp2* mutation does not likely affect the STA. However, if it does, we could not detect it with this set of experiments.

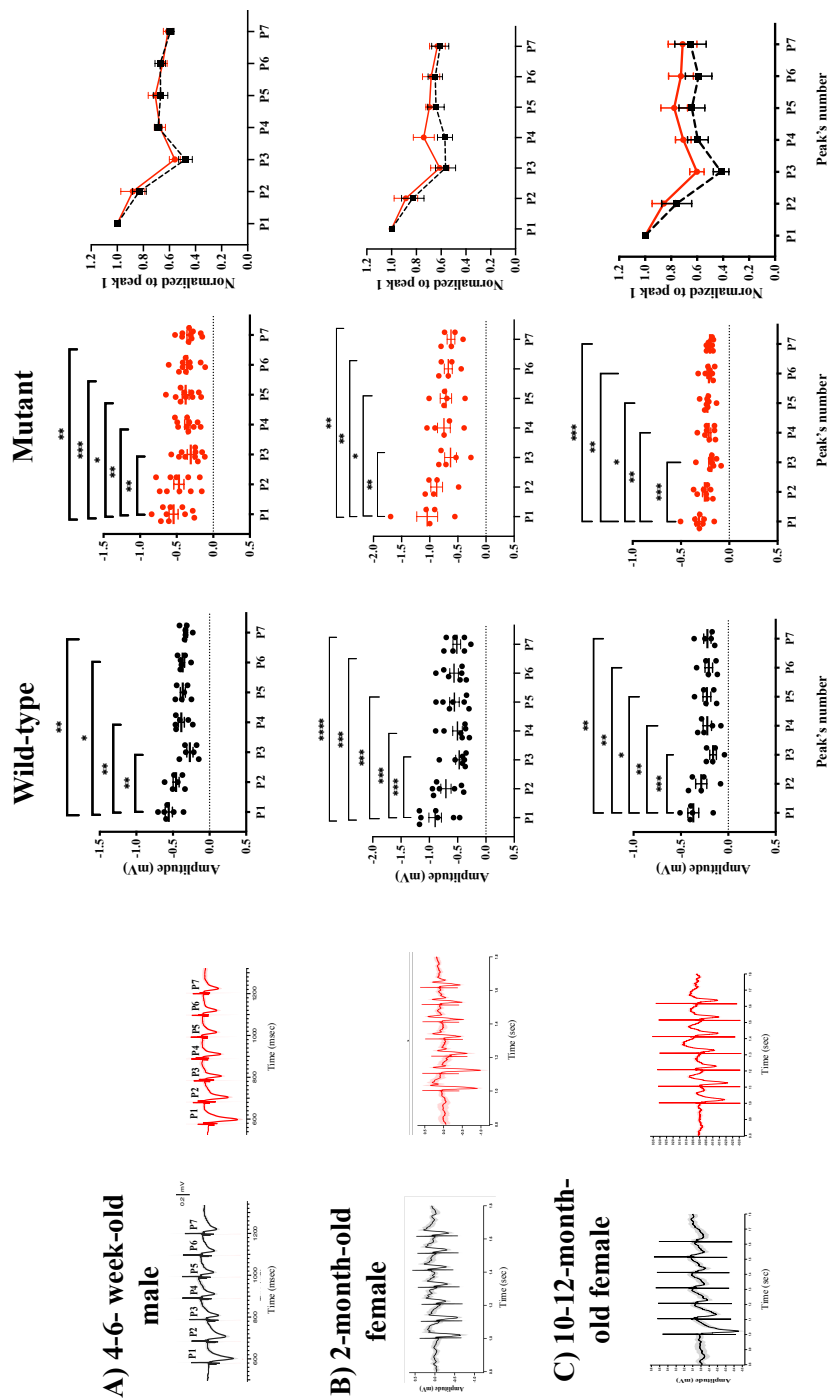


Figure 34, Short-term adaptation (STA) is intact in *Mecp2* mutant mice.

Traces show examples of brain evoked response to seven stimulation with 100ms interval (black= wild-type, red= *Mecp2* mutant). **A)** In male groups, the amplitude of subsequence peaks reduced compare to peak one in WT (n=6) mice ($p=0.0004$) and RTT(n=10) mice ($p<0.0001$). STA pattern was identical between WT and RTT groups ($p=0.8702$). **B)** In the 2-month-old female groups, STA was seen in both WT-YF ($p<0.0001$) and RTT-YF, ($p=0.0012$). The decline was similar between these groups ($p=0.7172$). **C)** In the older groups, both WT-F ($p=0.0211$) and RTT-F ($p=0.0093$) showed STA with a similar pattern. ($p=0.8371$). * $p<0.05$, ** $p<0.01$, *** $p<0.001$

3.4.6 The impact of short-term adaptation on the persistent sensory adaptation

Since the decline in the magnitude of peak response started on subsequence peaks and not P1, we hypothesized that the observed sensory adaptation (PA) results from increasing the STA in the sensory pathway and the recovery could potentially be due to a release from it. To test this hypothesis, the STA was graphed at the end of the adaptation and recovery phases for all the experimental groups.

At 60 minutes of adaptation phase:

In 4-6-week old male groups, the STA was still presented in both WT (n=6) ($p = 0.0003$) and RTT (n=10) ($p=0.0005$) groups with a similar rate ($p=0.1321$) (Figure. 35A). Similar data was seen in the 2-month-old female groups; both groups, WT-YF ($p<0.0001$), RTT-YF ($p=0.0012$), showed STA with a relatively similar pattern ($p= 0.6271$) (Figure. 35C). In the 10-12-month-old female groups, STA was observed in WT-F ($p= 0.0207$) and RTT-F ($p<0.0001$) at the degree ($p=0.0822$) (Figure. 35B). Together, the rate of STA was similar between *Mecp2* mutant and age-matched wild-type groups.

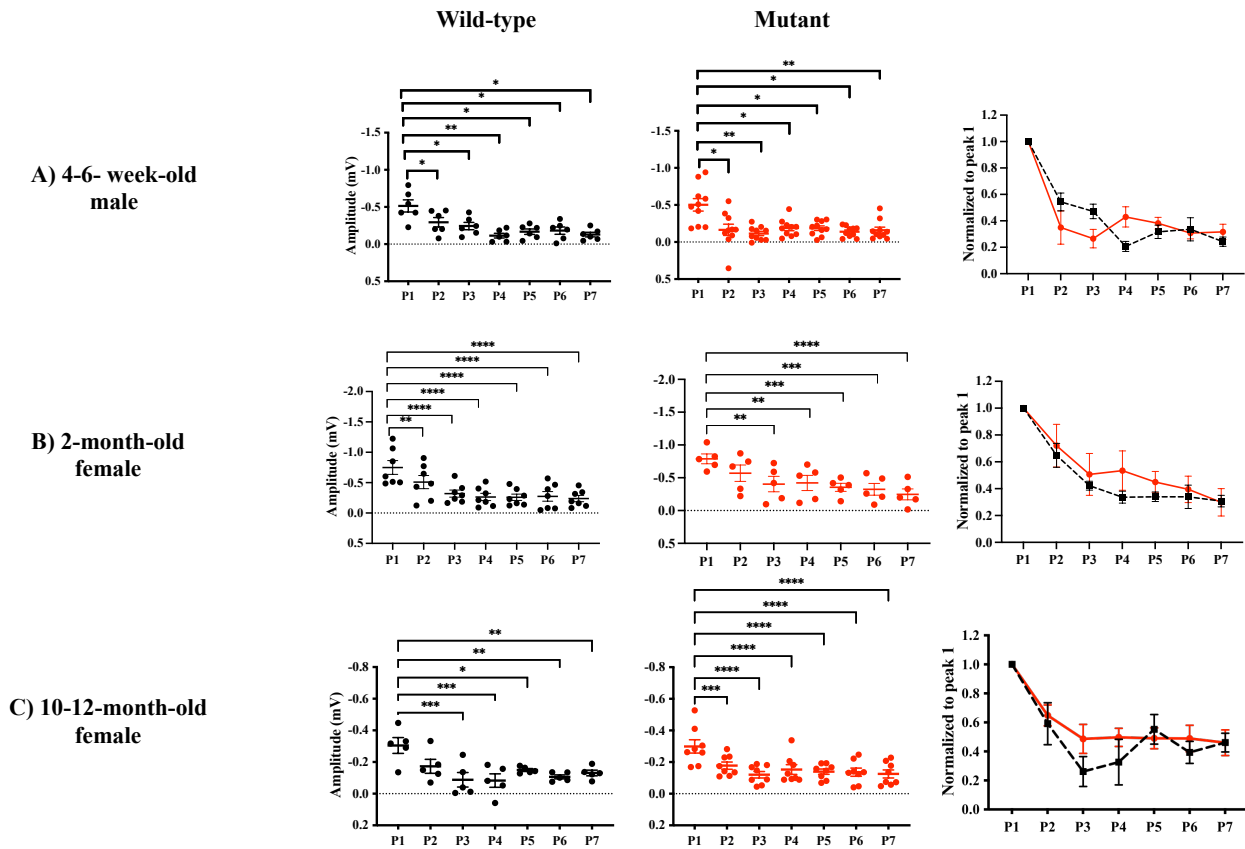


Figure 35, Synaptic short-term adaptation (STA) pattern is similar between Rett and age-matched wild-type mice after 60 minutes of repetitive HL stimulation.

A) In the 4-6-weeks-old male group, after 60 minutes of HL stimulation (100 Hz, ISI 6 sec), STA was still present in both WT ($p = 0.0003$) and RTT ($p=0.0005$) groups at the same rate ($P=0.1321$). **B)** In the 2-month-old female group, STA was observed in WT-YF ($n=7$) ($p<0.0001$) and RTT-YF ($n=5$) ($p=0.0012$). The rate of short-term adaptation was similar between WT-YF and RTT-YF ($p=0.6271$). **C)** In the 10-12-month-old female group, at the end of the adaptation phase (AP4), The reduction in P2 to P7 compare to P1 was observed. In WT-F ($p=0.0207$) and RTT-F ($p<0.0001$) with the same rate ($p=0.0822$).

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Comparing the STA degree at the 60 min of adaptation with the pre-stimuli indicated the increase of STA degree in all the young groups, which is more pronounced at the later peaks (P4, P5, P6 and P7) (Figure. 36A& B). In older female groups, none of the groups show a significant increase in the level of STA compare to baseline, which suggests that aging results in reduction of plasticity. However, in the RTT-F group, P5, P6, and P7 show more drop at 60 min of adaptation phase (Figure. 36C).

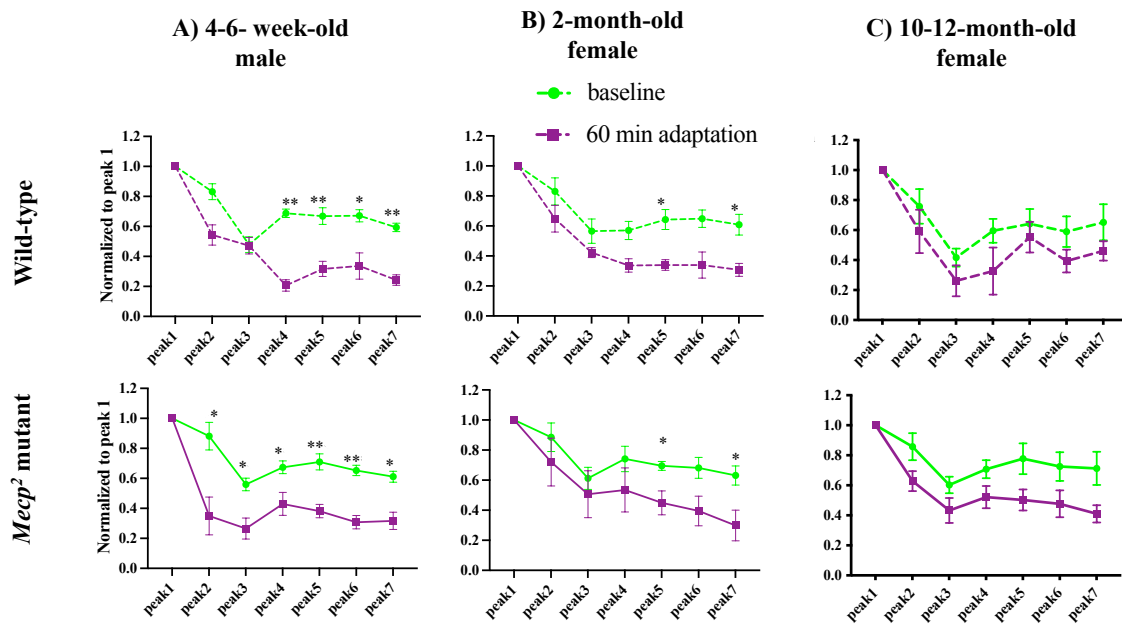


Figure 36, Short-term adaptation enhances after 60 minutes of HL stimulation

Peaks amplitudes are normalized to peak1 amplitude

A) In the 4-6-week-old male group, after 60minutes of HL stimulation (100 Hz, ISI 6 sec), more STA was observed in both WT ($p=0.0015$) and RTT ($p<0.0001$) compare to pre-stimuli. **B)** In 2-month old female group, STA was observed in WT-YF ($n=7$) ($p=0.0046$) and RTT-YF ($n=5$) ($p=0.2034$). **C)** In 10-12-month old females, at the end of the adaptation phase, the change in the STA degree was not significant from the baseline in neither WT-F ($p=0.06$) or RTT-F ($p=0.07$).

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Altogether, data suggest that STA enhancement could be potentially the cause of adaptation due to repetitive HL stimulation for 1 hour. The growth in the STA rate is similar between *Mecp2* mutant and age-matched groups, which is not surprising since the trend of the observed decline of sensory adaptation is similar between age-matched groups.

Since the increase of STA could be the reason for observed adaptation, does the fast recovery in *Mecp2* mutant result from decrease in STA? To answer this question, the rate of STA was calculated at 60 min of recovery.

At 60 minutes of recovery phase (rec4):

In 4-6-week-old male groups, the reduction in amplitude of subsequent peaks (P2 to P7) compared to P1 was observed in both WT ($p=0.0057$) and RTT ($p<0.0001$) at the same rate ($p=0.8637$) (Figure. 37A). In the younger female group, the STA in WT-YF ($p<0.0001$) and RTT-YF ($p=0.0002$) was not significantly different ($p=0.1341$) (Figure. 37B). However, the later peaks (P5, 6, and 7) exhibit less STA in RTT-YF than WT-YF. In older female groups, WT-F ($p=0.0221$) and RTT-F ($p=0.0005$) the similar rate of STA ($p=0.4050$) was seen (Figure. 37C).

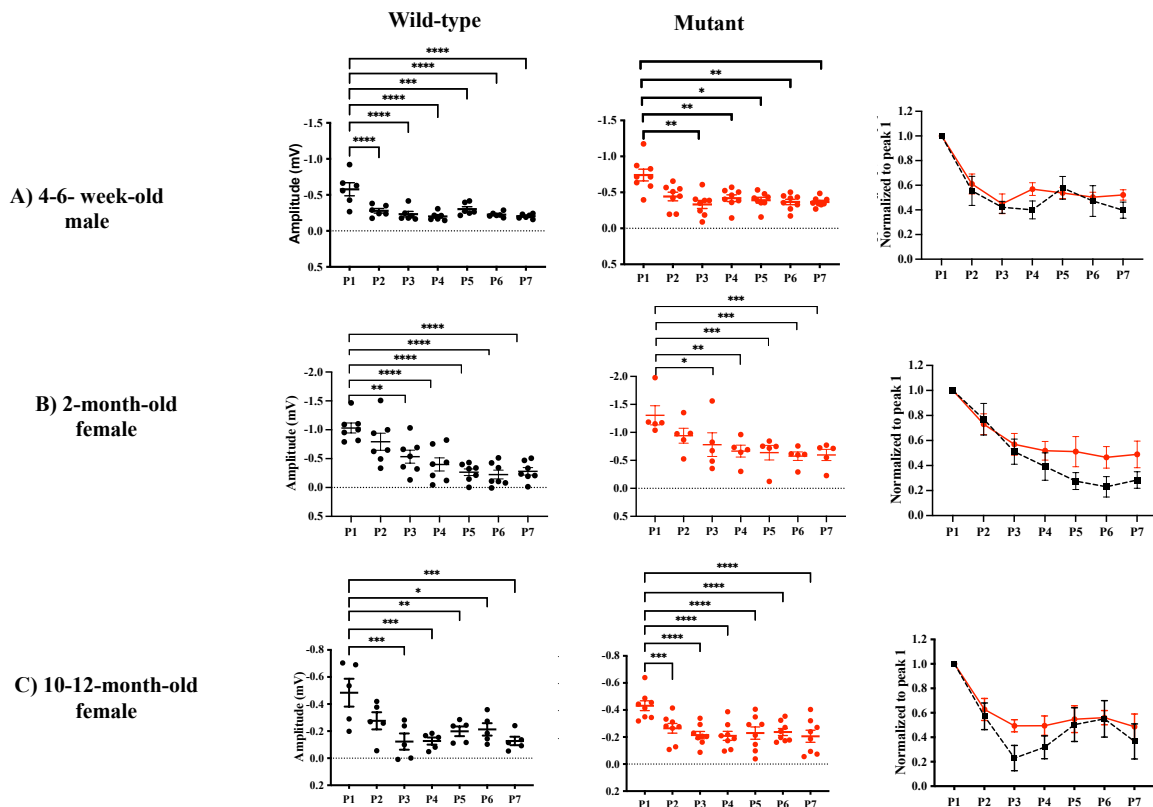


Figure 37, The trend of short-term adaptation (STA) is similar between wild-type and age-matched Rett model mice after 60 minutes of recovery following a sensory adaptation.

A) In 4-6-week old male groups, the reduction in the magnitude of peaks as a result of STA was observed in wild-type ($p=0.0057$) and Rett ($p<0.0001$) at the same degree ($p=0.8637$). **B)** 2-month-old WT-YF ($p<0.0001$) and RTT-YF ($p=0.0002$) showed the same degree of STA ($p=0.1341$). **C)** In 10-12-month-old female group, STA was still present in WT-F ($p=0.0221$) and RTT-F ($p=0.0005$). The rate of the change in the response magnitude of subsequent peaks was similar between WT-F and RTT-F ($p=0.4050$). $p=0.8637$). * $p<0.05$, ** $p<0.01$, *** $p<0.001$

To see whether the STA rate is changed after 60 min of recovery, the STA at 60 min of recovery was compared to the baseline and 60 min of adaptation. There is a slight decrease in the STA degree in both WT and RTT male group, meaning the STA reduced after 60 min of recovery compare to 60 min of adaptation but it did not reach to the baseline level (Figure. 38A). In young female, WT-F did not show any change in the STA degree from 60 min of adaptation to 60 min of recovery, which could be the reason for persistent adaptation in this group. In RTT-YF, the STA degree decreased a bit but similar to the male groups did not reach to the baseline level (Figure. 38B). There is no significant difference in the STA level between baseline, 60 min adaptation and 60 min of recovery in older groups (10-12-month old) (Figure. 38C).

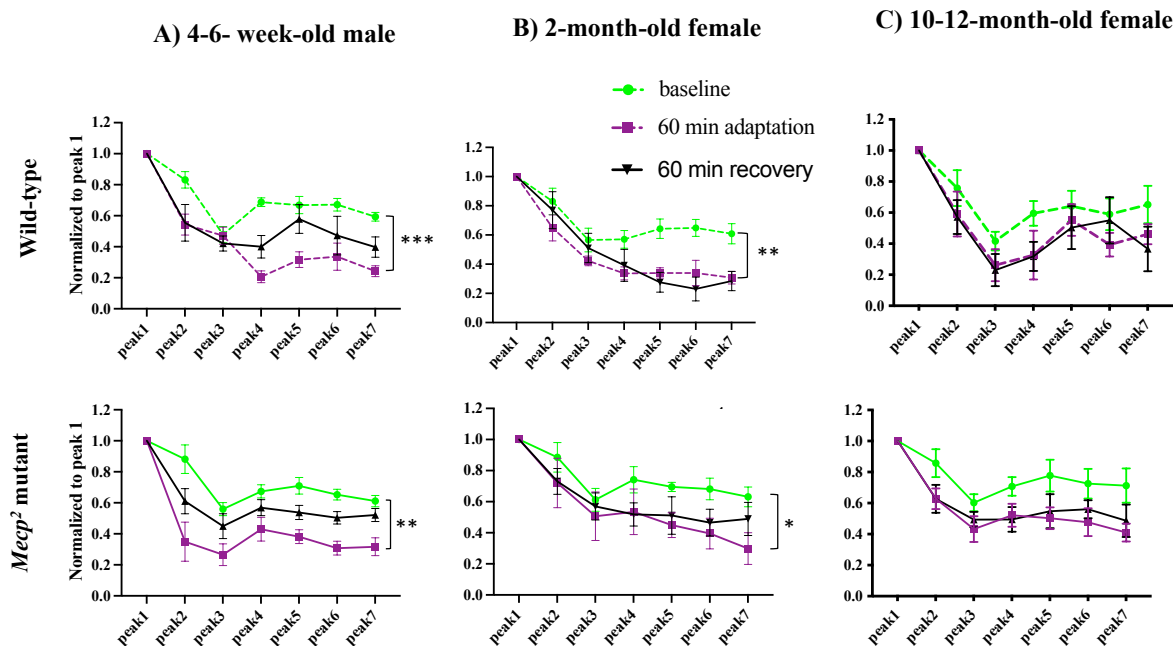


Figure 38, Change in the short-term adaptation (STA) level during the pre-adaptation, adaptation, and recovery phases

A) WT and RTT males show a moderate decrease in STA at the end of the recovery phase compared to 60 min of adaptation. However, it is still more than baseline. **B)** Although STA level at the end of recovery is mildly less than the 60 min of adaptation in 2-month old RTT-YF, it is the same in WT-YF. **C)** In 10-12-month old females, the STA level did not change. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Comparing the STA at the baseline, adaptation, and recovery phases showed the more pronounced change in the later peaks' amplitude (P5, 6, and 7). To improve the resolution of STA, we focused on the degree of STA at P5-7, summed them, and normalized it to the baseline (pre-adaptation) values (Figure. 39).

At 60 min of adaptation, STA was enhanced to the maximum; however, older groups showed a lower degree of STA enhancement than the rest. Thus, It suggests that there might be other mechanisms in the older animals that resist increasing the synaptic adaptation, which could result from a change in the sensory processing due to aging (Strömmer et al., 2017) or the smaller initial response (David-Jürgens & Dinse, 2010).

During the recovery phase, loss of increase in STA was observed in almost all the groups except the 2-month-old WT females. This could be a sign of generating more local inhibition during the adaptation phase, which results in long-lasting short-term adaptation in the circuit.

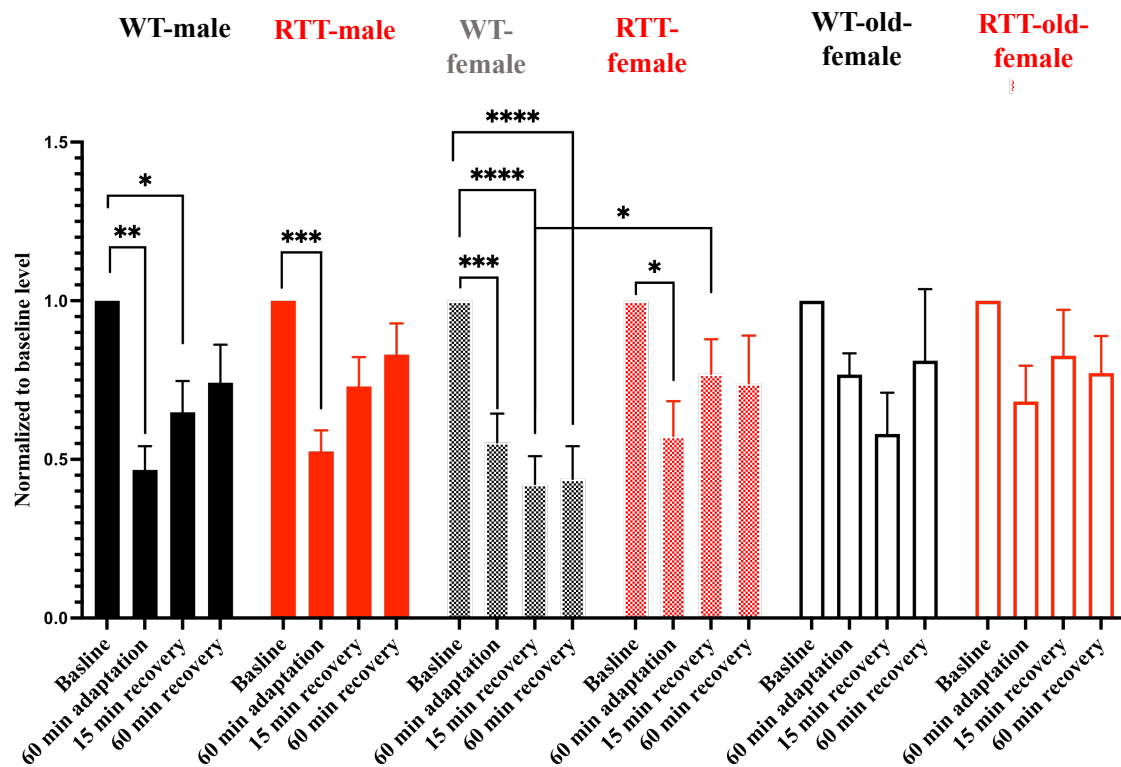


Figure 39, Loss of short-term adaptation during the recovery phase

The graph shows the short-term adaptation (STA) of peaks 5,6,7 at the end of the adaptation phase and during the recovery relative to the pre-adaptation phase.

Male groups: The same amount of STA enhancement at the 60min adaptation between WT ($p=0.0063$) and RTT ($p=0.0009$), which is not persistent in either of them during the recovery phases.

Young-females (pre-symptomatic): Although there is an increase in STA degree in both WT-YF ($p<0.0001$) and RTT-YF ($p=0.0177$) at 60 min of adaptation, Rett animals showed loss of STA during the recovery phase.

Old-females (symptomatic): Although the degree of STA enhancement in old groups was less than younger, it is slightly more persistent in WT-F

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Although data indicated that STA after 60 min of recovery is proportionally similar between WT and age-matched RTT mice groups, the loss of enhanced STA does not occur entirely in the mutant group at the end of the recovery period. Therefore, the faster recovery in the RTT group is not simply the result of loss of STA. Another factor that affects the magnitude of SER (sum of 7 peaks) is peak1. In the following section, we will discuss the changes in the P1 amplitude during the pre-adaptation, adaptation, and recovery phases in all the different experimental groups.

3.4.7 In *Mecp2* mutant model mice, the brain is at an excitable stage following repetitive hindlimb stimuli

In this set of experiments, seven sensory stimuli were applied. Referring to our data, there is mostly no change 1st peak amplitude during the adaptation period in any of the WT or age-matched mutant mice (Figure. 40A). When the feedback signaling was activated in the circuit, due to multiple sensory stimuli of HL (seven stimulations-100ms ISI), the reduction of cortical SER was noticeable (Figure. 40B). This trend was seen in both male and female groups.

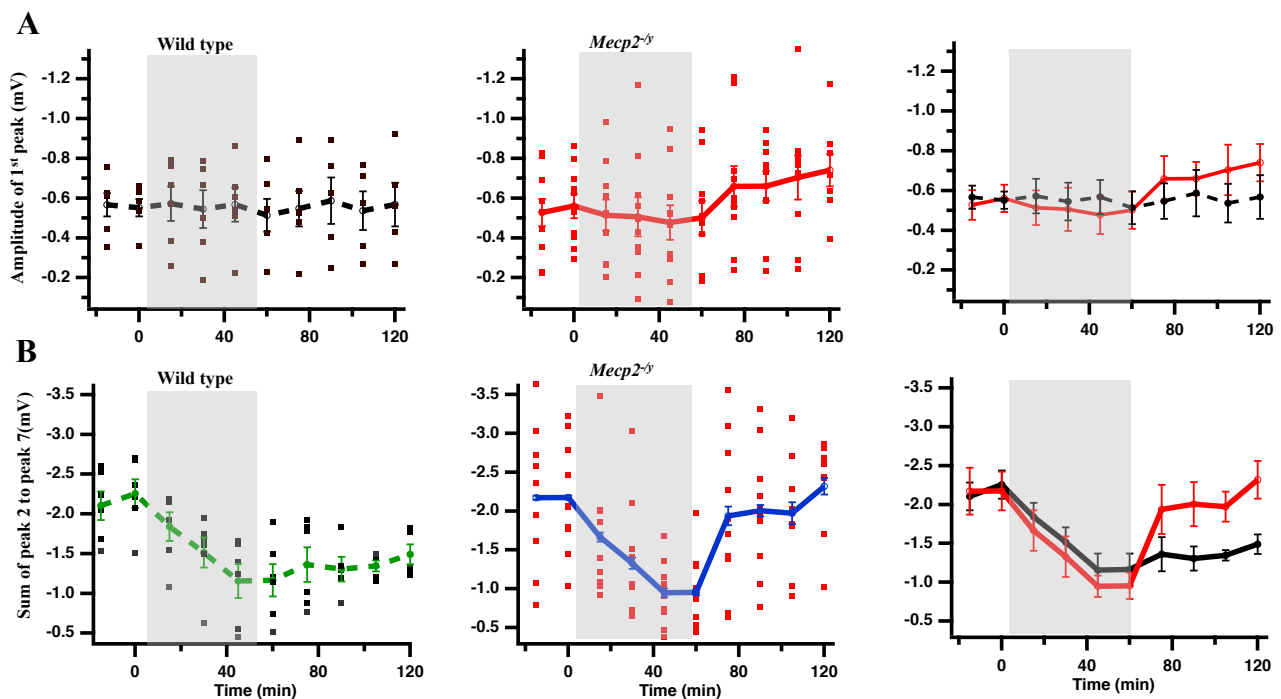


Figure 40, Drop of the cortical response in the adaptation phase was seen following the feedback network activation

A) The amplitude of peak one for 2-month-old male WT (left) and age-matched RTT (center) during the adaptation phase. **B)** Sum of peak two to seven in both WT (left) and age-matched RTT in the adaptation phase.

To investigate the P1 trend during the experiment, we normalized the P1 amplitude during the recovery time to the P1 amplitude prior to the adaptation (baseline) phase (Figure. 41).

Although the P1 magnitude was mostly stable during the adaptation phase, a minimal drop was seen at the 60 minutes of adaptation in all the groups (Figure. 41B) except the RTT-F (10-12-month old female) (Figure. 41B, right). It suggests that the excitability is suppressed at 60 minutes of adaptation along the HL to corresponding S1 due to the repetitive vibratory stimulation. At 15 minutes of recovery phase, the excitability returned to the baseline level in all the wild-type animals, while it was above the baseline in the mutant groups. In the male groups, at the end of recovery phase the excitability is significantly higher in the RTT group (hyperexcitability), which could be the main reason for fast recovery ($p=0.089$). In younger female groups, the excitability in mutant rose more than wild-type (Figure. 41B).

To study the rate of loss of suppression in the cortical excitability during the recovery phase compare to the end of adaptation, the P1 amplitude during the recovery was normalized to P1 amplitude at the 60 minutes of adaptation (Figure. 41C) In 4-6-week old male groups, gain of excitability was significantly higher in RTT than WT ($p=0.0272$) (Figure. 41C, left). Similarly, in 2-month-old groups, the same trend in loss of suppressed excitability in RTT-YF. There was a slight growth in the WT-YF which was significantly less than RTT-YF ($p=0.04$) (Figure. 41C, center). Opposite of the younger WT groups, the 10-12-month old female groups exhibited the increase of excitability during the adaptation phase which was same as RTT-F (Figure. 41C, right). This trend was not seen in any of the sham groups (Figure. 41D).

Based on the data in the younger animals, it could be concluded that one hour of HL stimulation put the Rett animals' (both male and female) brains in an excitable stage (sensitization). As a result, the 1st peak amplitude increased at 15 min of recovery. Therefore,

the magnitude of subsequence peaks (P2 to P7) rose following the increase of the 1st peak amplitude. Thus, the sum of these peaks in RTT is higher than WT, which results in faster recovery in mutant mice. Our data suggest that the recovery in pre-symptomatic female Rett model mice is mostly a result of reduction in STA and in some extent increasing excitability, while it is due to hyperexcitability in Rett male model mice.

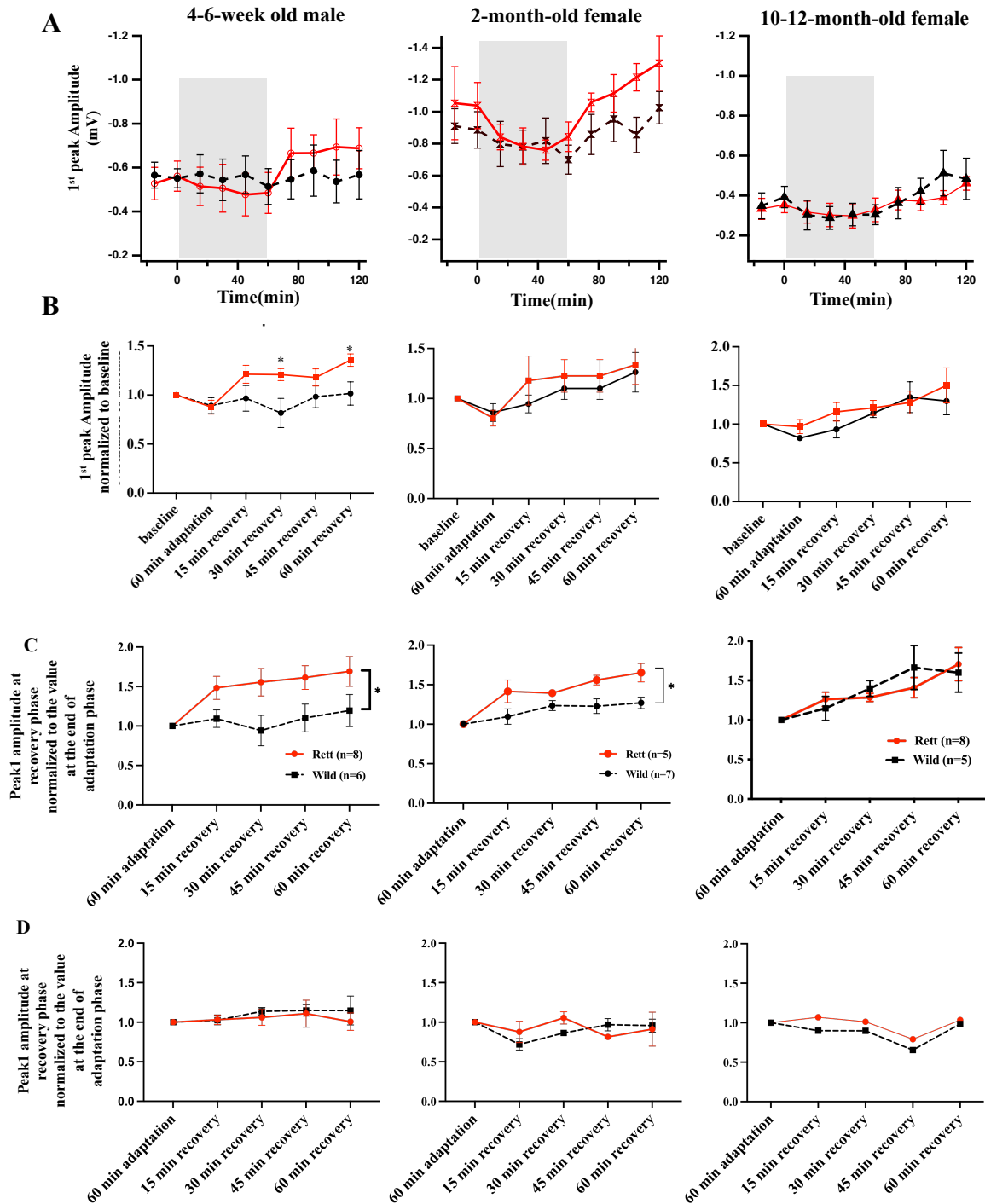


Figure 41, Brain is at an excitable level following repetitive hindlimb stimuli in young *Mecp2* mutant mice.

A) P1 amplitude during adaptation and recovery phases. **B)** Normalized the P1 amplitude during adaptation and recovery phases to P1 amplitude at the baseline. **C)** Normalized P1 amplitude from the recovery phase to the end of adaptation to show the excitability of the brain. Significant increase in *Mecp2*^{-/-} ($p=0.0272$) and 2-months-old *Mecp2*^{+/-} mice ($p=0.0266$) compared to age-matched groups and not in older female ($p=0.3523$). **D)** sham groups.

3.4.8 Peripheral sensory nerve recording

To investigate whether the observed sensory PA is central or peripheral, compound sensory nerve action potentials (SNAPs) from the sciatic nerve were recorded during the adaptation and recovery phases. There was no STA (Figure. 42 A, C & E) or sensory PA (Figure. 42 B, D & F) at the nerve ending level for any of the age/sex groups, indicating a central mechanism rather than the periphery.

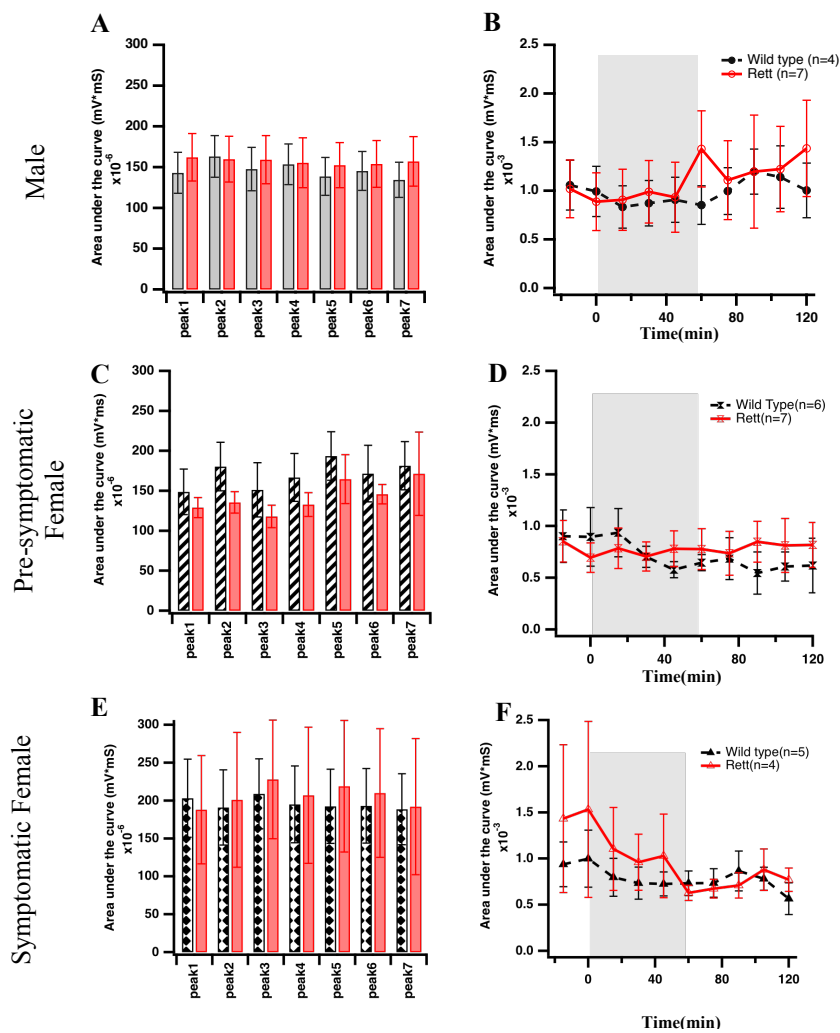


Figure 42, The sciatic nerve compound sensory nerve action potentials (SNAPs) to HL stimulation

SNAPs magnitude to 7 external sensory stimulation (100msec ISI) to monitor short-term adaptation in 4-6 weeks old male (A), pre-symptomatic female (C), and symptomatic female groups (E) at pre-adaptation period.

Sum of 7 SNAPs during adaptation and recovery phases in 4-6-week old male (B), pre-symptomatic (D), and symptomatic female groups (F).

3.4.9 Summary of the results

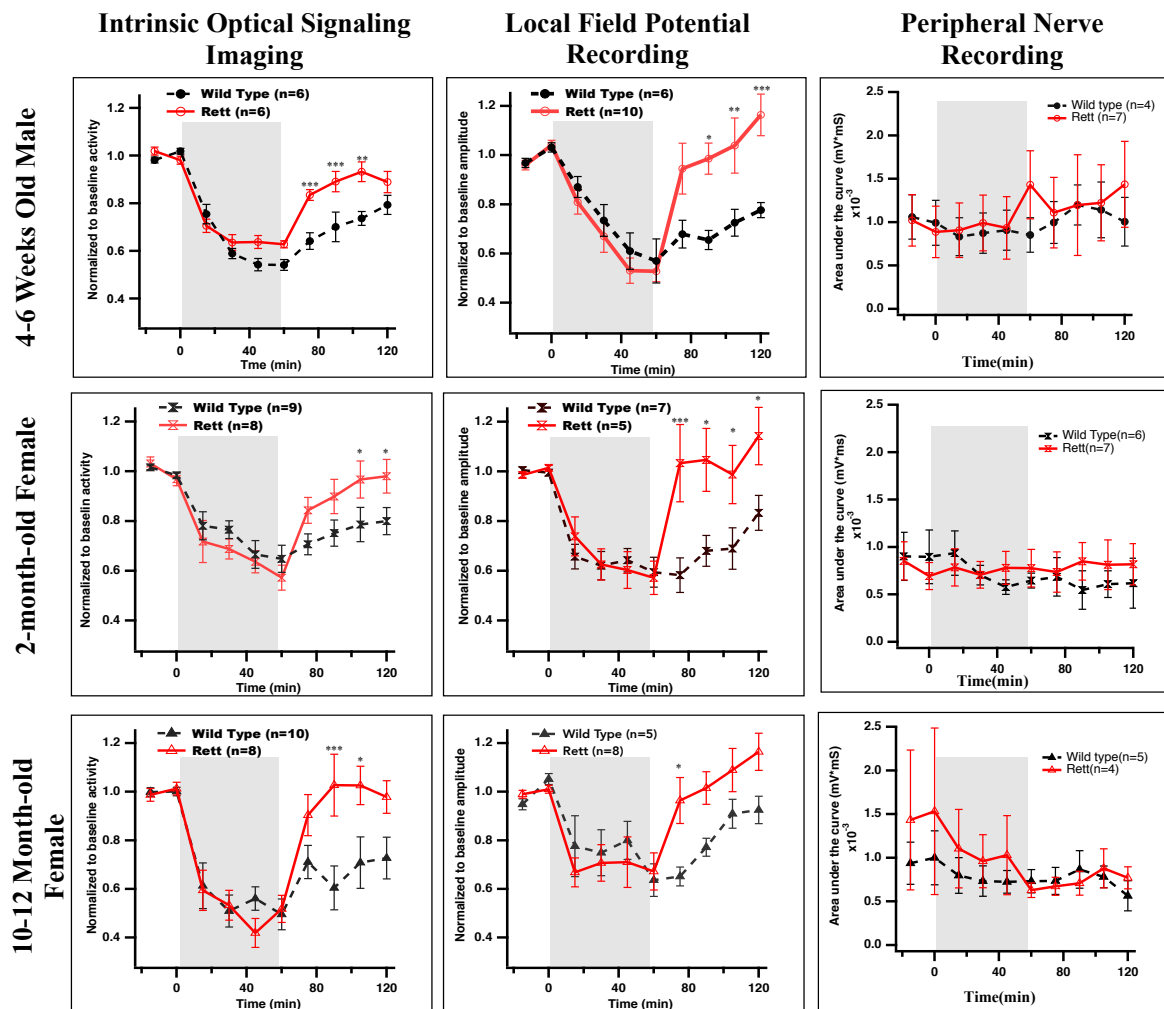


Figure 43, Summary of Results

Although, both wild type and Rett groups showed 40% reduction in cortical response due to repetitive hindlimb stimulation, Rett group showed a faster recovery. The observed cortical plasticity in the sensory pathway did not occur at peripheral ending.

Gray box shows the adaptation phase

3.5 Discussion

To our knowledge, this is the first study to evaluate sensory adaptation in the HL to somatosensory cortex pathway. Our data indicate that during an hour of continuous vibratory stimulation the tactile response of somatosensory neurons to a train of brief tactile stimuli is gradually diminish to the same degree in *Mecp2* mutant (RTT) and age-matched WT mice. After a period of rest the adaptation reverses only slowly in WT mice, with diminished response persisting (PA) for an hour more. However, in sharp contrast to WT the response to stimuli returns to at or near pre-adaptation level, typically within 30 minutes.

There are several synapses between HL and somatosensory cortex. Sensory afferent to medulla (gracile nucleus), medulla to thalamus, thalamus to cortex, and there are feedback pathways most notably cortex to thalamus, at which changes in transmission or excitability could occur (Figure. 44). Our direct measurements of sciatic nerve volleys suggest that at least the excitability of the primary afferents is not a primary site of diminish sensitivity. It has been shown in some instances that there is more pronounced adaptation at points downstream of the primary afferents. For example, VPM (thalamus) neurons show more adaptation in their responsiveness to sensory stimuli than the neurons in PrV (trigeminal (V) nucleus principals) (Sosnik et al., 2001; Ganmor et al., 2010) and less than cortical neurons (Sosnik et al., 2001; Khatri et al., 2009). The thalamocortical synapses undergo synaptic depression due to repetitive stimulation, which contributes to cortical adaptation (Chung et al., 2002).

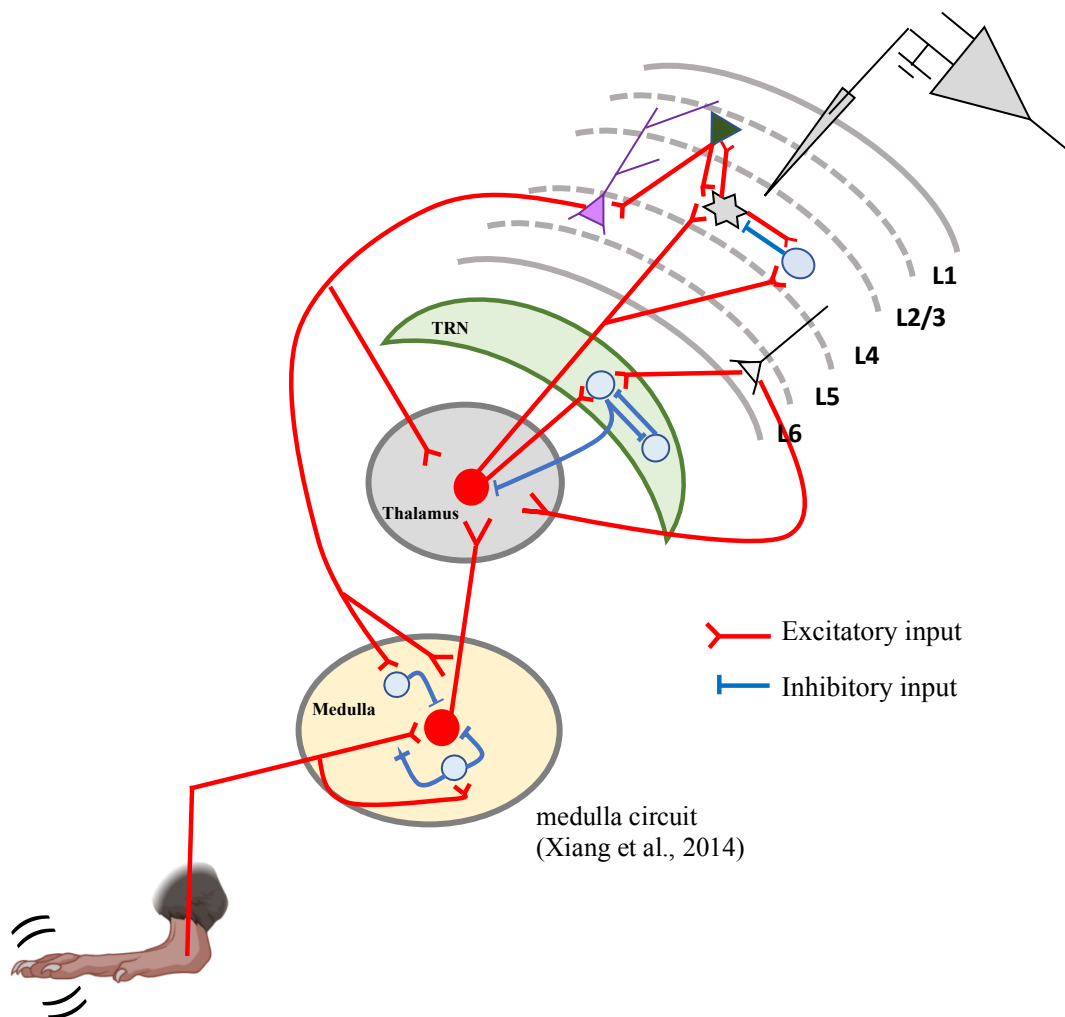


Figure 44, Hindlimb to primary somatosensory cortex, dorsal column-medial lemniscal, pathway and the recording electrode location

The initial response (peak1) at the cortex is due to feedforward signaling from HL to the corresponding somatosensory cortex (S1). Consequently, the cortex sends feedback signals to the thalamic reticular nucleus (TRN), thalamus, and brainstem, which will activate another circle of neuronal activity. S1 controls the ascending sensory information flow by sending feedback to the thalamus through corticothalamic projections. Projections from the cortex to the thalamus are through excitatory monosynaptic connections from mostly L6 or inhibitory di-synaptic via the TRN.

The 2nd response results from the integration of feedforward input from the HL and feedback signaling from the cortex. Peak 3 response is the cortical response to the 3rd stimulation, but the feedback activation modulates it due to the 2nd stimulation and the circuit activity due to the 1st stimuli. The same logic applies to the rest of the peaks, meaning that the circuit state modifies each response due to the earlier stimulation.

3.5.1 The impact of short-term synaptic adaptation in the persistent sensory adaptation

We assessed the tactile response in cortex based on the sum of response to a train of 7 single vibrations delivered at the intensity of 100 msec. Changes in the summed response could arise from changes in the amplitude of individual response and/or the degree of STA developing during the train. Referring to the figure 39 and 41, we saw evidence for changes in excitability as well as changes in the extent of short-term adaptation during the train to different degree in our male, young female and older female groups.

During the period of sustained vibration, the amplitude of the first response in both mutant and wild-type was relatively unchanged or slightly reduced but the summed response diminished due to substantially increased STA. During the recovery phase, PA was seen in the WT primarily because STA remained elevated as evidenced by decreased amplitude of the response to the 5th through 7th stimuli compared to the 1st. This suggest future studies could profitably look at enhanced feedback inhibitory pathway as a mechanism for PA.

The summed response in Rett male mice (RTT) returned quickly to baseline largely due to an increase in the amplitude of 1st response (increased excitability) along with to some degree reduction from enhanced STA compared to WT. While the STA in RTT remained slightly greater than baseline, to some extent this may reflect the increased response to the 1st stimuli. which in turn might be expected to generate more feedback inhibition and thus greater adaptation. However, we have not explored the relationship between stimuli intensity, initial response and rate of adaptation so whether the persistently enhanced STA seen in WT is absent in mutant cannot be determined. Regardless, the rapid return to response levels in mutant animals was characterized by evidence for further increasing the excitability following a period of sustained vibratory stimulation.

Compared to 4-6-week old males, 2-month old females showed even more dramatic retention of increased STA during the recovery (Figure. 39). Combined with a minimal of excitability (slightly increase in 1st peak amplitude) (Figure. 41) the result is persistently reduced summed response to the seven test stimuli. The mutant recovery to baseline responsiveness reflected a combination of much greater recovery of STA toward baseline and mild increasing of excitability (Figure. 39 & 41.).

The increase in the STA by the end of adaptation phase was less pronounced in the older Rett females (RTT-F). Results from RTT-F have to be considered in light of presence of clear motor and other symptoms but it is notable that the age-matched wild-type (WT-F) also showed less of an increase in STA, which could be due to smaller initial response (David-Jürgens & Dins, 2010), suggesting this form of plasticity exhibits an age dependence. As well, both WT-F and RTT-F showed similar slight, increase in excitability during the recovery period. Still, the faster recovery in RTT-F suggests the involvement of potentially another mechanism in the mutant group that influences the rate of recovery; however, we cannot address that in this project.

It is important to consider that symptom development in RTT males and old females may confine the ability to detect the difference of activity-dependent response between WT and mutant groups, while the healthy young *Mecp2*^{-/+} are more reliable. Our data indicated that the fast recovery in young RTT females is due to loss of STA and increased excitability.

3.5.2 Possible location of sensory adaptation in HL to S1 pathway

In the whisker-barrel cortex pathway, it has been suggested that short-term depression contributes to tactile adaptation at the cortex (Higley & Contreras, 2006; Heiss et al., 2008; Cohen-Kashi Malina et al., 2013), thalamus (Deschênes et al., 2003; Jubran et al., 2016), and brainstem (Mohar et al., 2015; Jubran et al., 2016). This reduction was greater at the cortical level, indicating that the cortical adaptation primarily results from the short-term depression of thalamocortical and intracortical synapses.

Since the synaptic depression has been observed in subcortical regions, we can conclude that the recorded cortical adaptation could be resulted from the STA cascade from HL to S1. In this study, we are not able to evaluate which of the synaptic relays from HL to S1 underwent STA. We know that the overall sensory adaptation is similar between RTT and age-matched WT. Still, we do not know if there is any difference in the degree of sensory adaptation at the individual synapses along the sensory pathway in these two groups. The difference in the rate of recovery between Rett and WT could be due to different sites of adaptation in the sensory pathway. It is clear that independent from the STA location in the sensory pathway; there are several differences between Rett and wild-type mice that could explain the observed dissimilarity in the recovery rate between these two groups. Although the site of synaptic adaptation might be the same or different, the consequence regarding the drop in cortical response is similar in RTT and WT. In addition, there might be only one site of adaptation in the sensory pathway of RTT animals. Still, because of the mutation of *Mecp2*, the result (drop in the cortical response) is the same as WT, which may have adaptation in more than one site, and therefore the recovery is longer.

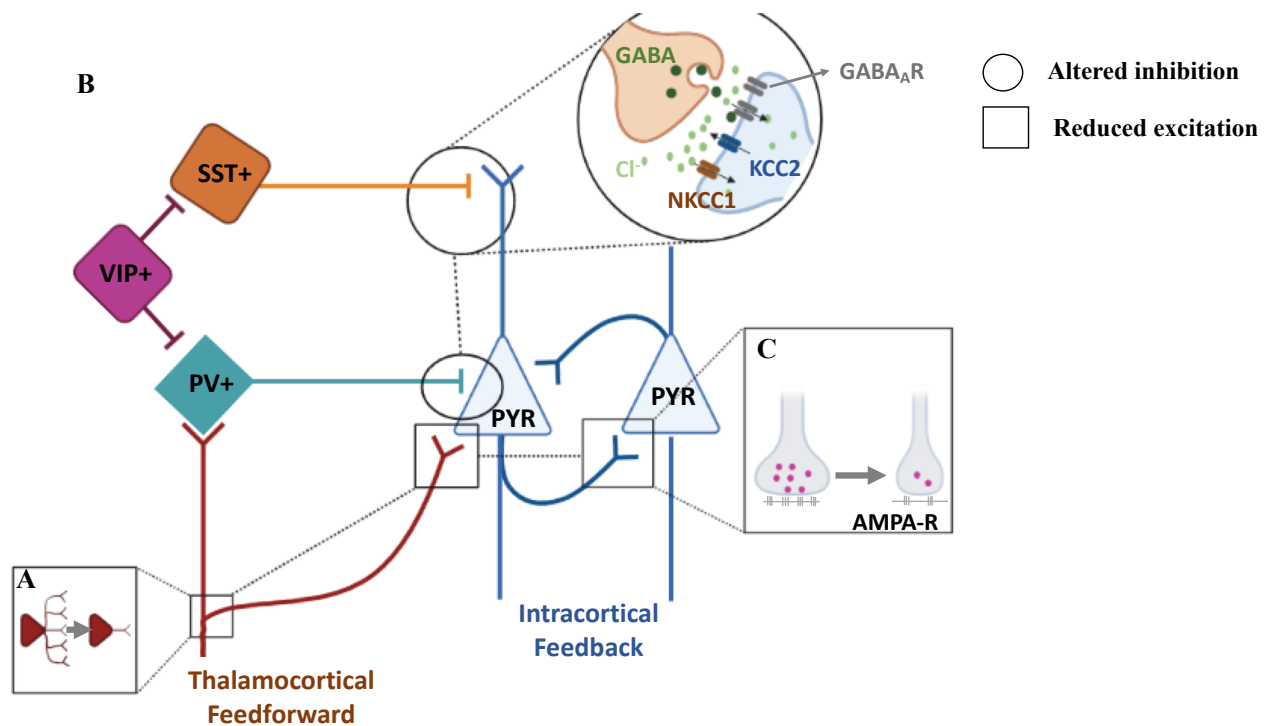


Figure 45, Altered excitation-inhibition in *Mecp2* mutant cortex *Mecp2* mutation

A) Thalamocortical feedforward excitatory drive is decreased in RTT due to a fewer number of synapses. B) Inhibitory current is reduced as a result of altered GABA_A receptor and reduction in KCC2 expression. C) Intracortical excitatory connection is reduced due to a decrease in the number of AMPA receptors.

SST= Somatostatin, VIP= Vasoactive intestinal polypeptide, PV= Parvalbumin

Intracortical level:

Overall sensory adaptation might result from adaptation at the intracortical, thalamocortical synapses or the level of the brainstem, individually, or it could be a sum of adaptation in all these different spots. Adaptation is not only the result of synaptic depression in the subcortical synapses; it also depends on cortical mechanisms (Chung et al., 2002).

Both excitatory and fast-spiking inhibitory neurons (PV⁺) in L4 of S1, receive sensory input through excitatory thalamocortical synapses from thalamus (Figure. 44). Thalamocortical synapses onto the PV⁺ neurons of somatosensory cortex are more robust than the ones onto the excitatory neurons in the same cortical layer (Cruikshank et al., 2007), and they depress more (Beierlein et al., 2003), shifting the balance of E/I toward excitation (Heiss et al., 2008). It is essential to notice, although the cortical E/I balance shifts toward excitation as a result of sensory adaptation, the depression of thalamocortical synapses leads to the reduction of excitation in the cortex, which prevents the cortical cells from reaching the spiking threshold in WT (Whitmire & Stanley, 2016) and results to decrease of net excitability. Altered inhibition (Banerjee et al., 2016) along with imbalance of cortical E/I has in *Mecp2* mutant mice (Dani et al., 2005; Lo et al., 2016; Calfa et al., 2015; Asgarihafshejani et al., 2019; Taneja et al., 2009; Abdala et al., 2010; Kron et al., 2012; Kron et al., 2012) could be in favor of more prolonged depression of cortical inhibition due to more hyper-connectivity of PV⁺ into pyramidal cells (Ip et al., 2018). Additionally, excitatory cells recover faster compare to inhibitory neurons (Cohen-Kashi et al., 2013). Therefore, after 15 minutes of the recovery phase, although the magnitude of sensory input to the cortex is unchanged (assuming adaptation occurs in the same rate for RTT and WT at the upstream synapses), the SER is higher in RTT because the inhibitory neurons are still depressed while excitatory ones are recovered and lead to a hypersensitivity following the release from adaptation (increase in the amplitude of peak1).

Thalamocortical level:

In *Mecp2* mutant mice, thalamocortical sensory-evoked feedforward drive is reduced due to a decrease in the number of synapses and an LTD-like mechanism at glutamatergic synapses (Banerjee et al., 2016b) (Figure. 45). Therefore, the thalamocortical input may not be depressed in RTT as much as WT; even the overall adaptation is the same. 15 minutes could be enough time for thalamocortical (thalamus to excitatory) synapse to recover and induce the large response in RTT groups since there are fewer synapses to recover (Figure. 45A).

Also, in RTT patients (Jian et al., 2007; Glaze et al., 2010; Cardoza et al., 2011) and *Mecp2* mutant mice (Calfa et al., 2011; McLeod et al., 2013), the neuronal spiking threshold is reduced. Therefore, simultaneous activation of a few inputs from the thalamus to the cortex is sufficient enough for the excitatory cells to reach the spiking threshold. This neuronal activity spreads quickly to the rest of the population, which is observed as an increased magnitude of P1. When the E/I balance is normal, more input is needed to bring the neurons to the firing threshold, which could be the reason for the observed delay in recovery in WT since there are more cortical afferents that need to recover from adaptation.

Although thalamic cells did not fully recover to the baseline level after sustain whisker stimulation in WT rat (Cohen-Kashi et al., 2013), they might recover completely in RTT.

There is also less GABAergic transmission due to *Mecp2* mutation in the thalamus which could be the reason for excitability and faster recovery in mutant groups (Zhang et al., 2010).

Brainstem level:

Synaptic hyper-excitability in brainstem areas (Taneja et al., 2009; Abdala et al., 2010; Kron et al., 2012) could be the reason for fast recovery. Since the medulla does not go under

adaptation as much as the cortex, the initial response going through the medulla to the thalamus in the mutant mice is greater. Therefore, even if the same amount of adaptation is present in the cortex at 60 min of adaptation phase, the larger input induce greater initial response.

overall, at 60 min of adaptation period, the net excitability is at the minimum level in both groups which could be due to enhanced inhibition or reduced excitation in the H1 to S1 pathway. Increase of inhibition results from facilitation of inhibitory synapse or depression of excitatory synapse, which could be the same or different between mutant and WT group. Regardless, the overall reduction in response is similar. The faster recovery in RTT is due to loss of enhanced STA and increased excitability which could be explained by the multiple differences in the sensory pathway.

3.5.3 Possible oversensitivity in *Mecp2* mutation

There is a significant difference in the magnitude of baseline SER, recorded by IOS imaging, in *Mecp2*^{fl/y} male compare to age-matched WT, which indicates a possible hypersensitivity due to *Mecp2* mutation in RTT model mice. In the pre-symptomatic females, there is a slight difference in the magnitude of baseline evoked response between RTT and WT animals in favor of RTT animals (RTT mice showed higher SER) which is more pronounced in LFP data. This variation in heterozygous *Mecp2*^{+/-} females result is due to skew of X-chromosome inactivation (Young et al., 2005) which causes the different degree of abnormality in this group.

A “giant”- somatosensory evoked potential (SEP) is observed in patients younger than nine years old, indicating cortical hyperexcitability in RTT patients (Yoshikawa et al., 1991). In RTT animals, hypersensitivity to a gentle touch due to the reduction of inhibitory synaptic drive in the spinal cord (Orefice et al., 2016) and an increase in N1 amplitude of visual evoked potential (VEP) have been reported (Liao et al., 2012). The hyperexcitability in RTT patients

and animal models could be due to altered excitatory threshold (Sysoeva et al., 2020) and less inhibition into the VPL since RTN makes fewer synapses to VPL in the *Mecp2* mutant mice (Z. W. Zhang et al., 2010). Additionally, It could result from impairment in the transmission of sensory signals from the thalamus to layer 4 of the somatosensory cortex due to increased efficiency of GABAA receptors in the barrel cortex (Lo et al., 2016). Synaptic hyperexcitability is observed in brainstem areas, including locus coeruleus (Taneja et al., 2009), ventrolateral medulla (Abdala et al., 2010), and nucleus tractus solitarius (Kron et al., 2012a).

The hyperexcitability was not seen in older RTT female animals (10-12 months old). Similar to our data, the giant SEP, which was seen in young patients, was not observed in older groups (>9 years old), which could be due to the resolve of clinical seizures in older individuals (Yoshikawa et al., 1991).

3.5.4 Reduced sensory evoked response magnitude due to ageing

In the wild-type group, the size of SER was reduced in older animals, which could be effect of aging on sensory processing of somatosensory and auditory stimuli (Strömmer et al., 2017). Similarly, in the Rett group, there was a negative correlation between age and response amplitude. Overall, the symptomatic female group (10-12-month old) showed a smaller SER amplitude than the pre-symptomatic one (2-month old). Same trend was seen in VEP amplitude, the visual cortical processing deficits are worsened by aging due to *Mecp2* mutation (LeBlanc et al., 2015). The reduction in the 10-12-month old group, aging is a second factor affecting the magnitude of the cortical SER to an external stimulus.

3.5.5 50% reduction in *Mecp2* expression is compelling enough for cortical plasticity impairment

Comparing the result between *Mecp2*^{-y} male and *Mecp2*^{+/-} female did not show any significant difference in the trend of adaptation and recovery. It was expected that due to the complete lack of MECP2 in the null mice, the effect be more pronounced in *Mecp2*^{-y} mice. Our data suggested a 50% reduction in *Mecp2* expression or, in some cases, even less, as unbalanced patterns of X-chromosome inactivation generally favor the expression of the wild-type allele (Young et al., 2005), is enough to alter the cortical plasticity in the heterozygous female mice. In addition, data showed that this disruption in the cortical plasticity is present in the sensory pathway at an early age (pre-symptomatic), suggesting that some of the cognitive impairment may result from the incapability of the *Mecp2* mutant brain to maintain the long-lasting changes in neuronal activity.

3.6 Conclusion

Our data indicated that repetitive stimulation of HL leads to sensory adaptation in both RTT and WT but a faster recovery in the RTT group. This reduction is more likely due to enhanced feedback inhibition or synaptic depression in synapses along the CNS in the sensory pathway from the HL to S1. Observed adaptation in the somatosensory cortex could be generated locally, or some of the components are inherited from the earlier stage to the next one in the pathway.

In conclusion, the fast recovery in mutant mice indicates that the *Mecp2* mutant is not able to maintain long-lasting changes in neuronal activity. This is another piece of evidence regarding the role of MECP2 in the maintenance of neuronal circuits.

Table 2: Statistical analysis of chapter 3 result

Figure	Sample size	Statistical test	Values
Fig23-A	WT (n=6)	RM ⁴ One-Way ANOVA Post-hoc: Tukey's test (Adaptation) Post-hoc: Tukey's test (Recovery)	F (9,45) =35.46, $p<0.0001$ 15 min: $p=0.0267$, 30min: $p=0.0001$, 45min: $p=0.0002$, 60min: $p<0.0001$ 75 min: $p=0.25$, 90min: $p=0.19$, 105min: $p=0.0015$, 120min: $p=0.007$,
Fig23-B	RTT (n=6)	RM One-Way ANOVA Post-hoc: Tukey's test (Adaptation) Post-hoc: Tukey's test (Recovery)	F (9,45) =27.46, $p<0.0001$ 15 min: $p=0.0042$, 30min: $p=0.0007$, 45min: $p<0.0001$, 60min: $p<0.0001$ 75 min: $p=0.0096$, 90min: $p=0.025$, 105min: $p=0.012$, 120min: $p=0.029$,
Fig23-C	both groups,	RM Two-Way ANOVA Post hoc: Sidak	F (9, 90) = 4.673, $P<0.0001$ Baseline: $p=0.0079$ 45min: $p=0.037$, 75min: $p=0.0001$, 90min: $p<0.0001$, 105min: $p<0.0001$, 120min: $p<0.0059$
Fig23-D	both groups, (n=6)	RM Two-Way ANOVA Post hoc: Sidak (Recovery)	F (9,90) = 5.272, $P<0.0001$ 75 min: $p=0.0001$, 90 min : $p=0.0001$ 105 min: $p=0.0004$
Fig24	both groups, (n=6)	RM Two-Way ANOVA effect of genotype	F (9,86) =1.031, $p=0.4223$ F (1,10) =33.31, $p=0.0002$
Fig25A	RTT(n=12) WT(n=11)	Unpaired t-test, 2 tailed	Test group base1: $t=2.454$, $df=21$, $p=0.029$ base2: $t=2.140$, $df=21$, $p=0.0442$
Fig25B	RTT (n=12) WT (n=11)	Unpaired t-test, 2 tails	base1: $t=3.941$, $df=21$, $p=0.0007$ base2: $t=4.273$, $df=21$, $p=0.0003$
Fig25C	RTT(n=24) WT (n=22)	Correlation co-efficiency	$R^2=0.01260$, $p=0.6$ $R^2=0.0234$, $p=0.49$
Fig26A	RTT-F (n=10) WT-F (n=8)	Un-paired t-test, 2-tailed	$p<0.0001$, $t=5.423$, $df=17$,
Fig27A	WT-F (n=8)	RM One-Way ANOVA Post-hoc: Tukey's test (adaptation)	F (9,63) =35.46, $p=0.0005$ 15 min: $p=0.038$, 30min: $p=0.0026$, 45min: $p=0.0051$, 60min: $p=0.0029$
Fig27B	RTT-F (n=10)	RM One-Way ANOVA Post-hoc: Tukey's test (adaptation)	F (9,81) =15.18, $p<0.0001$ 15 min: $p=0.056$, 30min: $p=0.006$, 45min: $p=0.0049$, 60min: $p=0.0012$

⁴ Repeated measure

		Post-hoc: Tukey's test (Recovery)	75 min: $p=0.0134$, 90min: $p=0.0291$, 105min: $p=0.0004$, 120min: $p=0.0012$,
Fig27C	WT-F (n=8) RTT-F (n=10)	RM Two-Way ANOVA Post hoc: Sidak	F (9,144) = 3.911, $p=0.0002$ 90min: $p=0.0007$ 105min: $p=0.0251$
Fig28	WT-YF (n=17) RTT-YF (n=16)	Un-paired t-test, 2-tailed	$t=1.438$, $df=31$, $p=0.26$
Fig29A	WT-YF (n=9)	RM One-Way ANOVA Post-hoc: Tukey's test (adaptation)	F (9, 72) = 9.593, $p=0.0001$ 15 min: $p=0.5$, 30min: $p=0.007$, 45min: $p=0.019$, 60min: $p=0.027$
Fig29B	RTT-YF, (n=8)	RM One-Way ANOVA Post-hoc: Tukey's test (adaptation) Post-hoc: Tukey's test (Recovery)	F (9,63) = 10.85, $p<0.0001$ 15 min: $p=0.14$, 30min: $p=0.007$, 45min: $p=0.0063$, 60min: $p=0.02$ 75 min: $p=0.05$, 90min: $p=0.07$, 105min: $p=0.026$, 120min: $p=0.010$
Fig.29C	WT-YF (n=9), RTT-YF (n=8)	RM Two-Way ANOVA	F (9,135) = 3.985, $p=0.0002$ 105min: $p=0.049$, 120min: $p=0.039$
Fig. 31C	WT(n=6)	RM One-Way-ANOVA Post-hoc: Tukey's test (adaptation)	F (9,45) = 11.66, $p=0.0004$ 15 min: $p=0.0692$, 30min: $p=0.0143$, 45min: $p=0.0041$, 60min: $p=0.0075$
Fig. 31D	RTT (n=10)	RM One-Way-ANOVA Post-hoc: Tukey's test (adaptation) Post-hoc: Tukey's test (recovery)	F (9,81) = 12.39, $p<0.0001$ 15 min: $p=0.0195$, 30min: $p=0.0178$, 45min: $p=0.0015$, 60min: $p=0.0005$ 75 min: $p=0.0265$, 90min: $p=0.0017$, 105min: $p=0.0262$, 120min: $p=0.0115$
Fig. 31E	WT, (n=6), RTT (n=10)	RM Two-Way ANOVA, Post hoc: Sidak	F (9,126) = 5.028, $p<0.0001$ 90 min: $p=0.0142$, 105min: $p=0.02$, 120min: $p=0.0098$
Fig. 32C	WT-F (n=5)	RM One-Way-ANOVA Post hoc: Sidak	Friedman statistic=25.47, $p=0.0025$ 60min: $p=0.0044$, 70 min 0.0124
Fig. 32D	RTT-F (n=8)	RM One-Way ANOVA Post-hoc: Sidak (adaptation) Post-hoc: Sidak (recovery)	F (9,63) = 15.1, $p<0.0001$ 15 min: $p=0.0002$, 30min: $p=0.0021$, 45min: $p=0.0010$, 60min: $p=0.0002$ 75 min: $p=0.001$, 90min: $p=0.0002$, 105min: $p<0.0001$, 120min: $p<0.0001$

Fig. 32E	WT-F (n=5) RTT-F (n=8)	RM Two-Way ANOVA, Post hoc: Sidak	F (9,99) =4.022, $p=0.0002$ 90 min: $p=0.04$
Fig. 33C	WT-YF (n=7)	RM One-Way ANOVA, Post-hoc: Sidak (adaptation)	F (9, 54) =14.14, $p<0.0001$ 15 min: $p<0.0001$, 30min: $p<0.0001$, 45min: $p<0.0001$, 60min: $p<0.0001$
Fig. 33D	RTT-YF (n=5)	RM One-Way ANOVA Post-hoc: Tukey's test (PA) Post-hoc: Tukey's test (recovery)	F (9,63) =15.1, $p<0.0001$ 15 min: $p=0.055$, 30min: $p=0.0088$, 45min: $p=0.0030$, 60min: $p=0.0026$ 75 min: $p=0.023$, 90min: $p=0.0127$, 105min: $p<0.0293$, 120min: $p=0.0004$
Fig. 33E	WT-YF (n=7) RTT-YF (n=5)	RM Two-Way ANOVA Post-hoc: Sidak (recovery)	F (9,90) =5.212, $p<0.0001$ 75 min: $p=0.0006$, 90 min: $p=0.0102$, 105 min: $p=0.0467$, 120 min, $p=0.0472$
Fig. 34A	WT (n=6)	RM One-Way-ANOVA Post-hoc: Tukey's test	F (6,30) =16.12, $p=0.0004$ P1 vs. P2: $p=0.113$ P1 vs. P3: $p=0.003$ P1 vs. P4: $p=0.0021$ P1 vs. P5: $p=0.067$ P1 vs. P6: $p=0.0252$ P1 vs. P7: $p=0.0079$
	RTT (n=10)	RM One-Way-ANOVA Post-hoc: Tukey's test	F (6,54) = 13.17, $p<0.0001$ P1 vs. P2: $p=0.3033$ P1 vs. P3: $p<0.0001$ P1 vs. P4: $p<0.0001$ P1 vs. P5: $p=0.0001$ P1 vs. P6: $p<0.0001$ P1 vs. P7: $p<0.0001$
	normalized	RM Two-Way ANOVA	F (6,84) =0.3728, $p=0.8943$
Fig. 34B	WT-YF (n=7)	RM One-Way ANOVA Post-hoc: Tukey's test	F(6,36)=9.54, $p<0.0001$ P1 vs. P2: $p=0.113$ P1 vs. P3: $p<0.0001$ P1 vs. P4: $p<0.0001$ P1 vs. P5: $p=0.0003$ P1 vs. P6: $p=0.0005$ P1 vs. P7: $p<0.0001$
	RTT-YF (n=5)	RM One-Way ANOVA,	F(6,24)=5.406, $p=0.0012$ P1 vs. P2: $p=0.55$ P1 vs. P3: $p=0.028$ P1 vs. P4: $p=0.053$ P1 vs. P5: $p=0.022$ P1 vs. P6: $p=0.008$ P1 vs. P7: $p=0.0023$
	normalized	RM Two-Way ANOVA	F(6,60)= 0.7302, $p=0.7172$
Fig. 34C	WT-F (n=5)	RM One-Way ANOVA Post-hoc: Tukey's test	F(7,24)=6.486, $p=0.0004$ P1 vs. P2: $p=0.3109$

			P1 vs. P3: $p=0.0002$ P1 vs. P4: $p=0.0091$ P1 vs. P5: $p=0.012$ P1 vs. P6: $p=0.0033$ P1 vs. P7: $p=0.0087$
	RTT-F	RM One-Way ANOVA Post-hoc: Tukey's test	$F(7,42)=6.490, p=0.0001$ P1 vs. P2: $p=0.2145$ P1 vs. P3: $p=0.0001$ P1 vs. P4: $p=0.0041$ P1 vs. P5: $p=0.0168$ P1 vs. P6: $p=0.0031$ P1 vs. P7: $p=0.0007$
	normalized	RM Two-Way ANOVA	$F(6,66)=0.4575, p=0.8371$
Fig. 35A	WT (n=6)	RM One-Way-ANOVA Post-hoc: Tukey's test	$F(5,30)=20.13, p=0.0003$ P1 vs. P2: $p=0.017$ P1 vs. P3: $p=0.045$ P1 vs. P4: $p=0.007$ P1 vs. P5: $p=0.015$ P1 vs. P6: $p=0.028$ P1 vs. P7: $p=0.012$
	RTT (n=10)	RM One-Way-ANOVA Post-hoc: Tukey's test	$F(6,54)=13.84, p<0.0001$ P1 vs. P2: $p=0.0048$ P1 vs. P3: $p=0.005$ P1 vs. P4: $p=0.011$ P1 vs. P5: $p=0.011$ P1 vs. P6: $p=0.016$ P1 vs. P7: $p=0.009$
	together	RM Two Way-ANOVA	$F(6,84)=2.549, P=0.025$
Fig. 35B	WT-YF (n=7)	RM One-Way ANOVA Post-hoc: Tukey's test	$F(6,36)=22.47, p<0.0001$ P1 vs. P2: $p=0.0025$ P1 vs. P3: $p<0.0001$ P1 vs. P4: $p<0.0001$ P1 vs. P5: $p<0.0001$ P1 vs. P6: $p<0.0001$ P1 vs. P7: $p<0.0001$
	RTT-YF (n=5)	RM One-Way ANOVA,	$F(6,24)=10.02, p<0.0001$ P1 vs. P2: $p=0.139$ P1 vs. P3: $p=0.0014$ P1 vs. P4: $p=0.0023$ P1 vs. P5: $p=0.0003$ P1 vs. P6: $p=0.0001$ P1 vs. P7: $p<0.0001$
	normalized	RM Two-Way ANOVA	$F(6,60)=10.02, p=0.62$
Fig. 35C	WT-F (n=5)	RM One-Way ANOVA	$F(6,24)=6.095, P=0.0005$ P1 vs. P2: $p=0.0767$ P1 vs. P3: $p=0.0008$ P1 vs. P4: $p=0.0006$ P1 vs. P5: $p=0.024$ P1 vs. P6: $p=0.0023$

			P1 vs. P7: $p=0.009$
	RTT-F (n=5)	RM One-Way ANOVA	F(6,42)=12.3, $p<0.0001$ P1 vs. P2: $p=0.0004$ P1 vs. P3: $p<0.0001$ P1 vs. P4: $p<0.0001$ P1 vs. P5: $p<0.0001$ P1 vs. P6: $p<0.0001$ P1 vs. P7: $p<0.0001$
	normalized	RM Two-Way ANOVA	F(6,66)=1.029, $P=0.4145$
Fig. 36A	Male (WT) (n=6)	Multiple paired t-test	P2: $p=0.091$, $t=2.624$, $df=5$ P3: $p=0.90$, $t=0.1218$, $df=5$ P4: $p=0.0007$, $t=10.64$, $df=5$ P5: $p=0.006$, $t=6.267$, $df=5$ P6: $p=0.04$, $t=3.633$, $df=5$ P7: $p=0.003$, $t=7.501$, $df=5$
Fig. 36A	Male (RTT) (n=10)	Multiple paired t-test	P2: $p=0.01$, $t=3.762$, $df=9$ P3: $p=0.01$, $t=3.965$, $df=9$ P4: $p=0.01$, $t=4.103$, $df=9$ P5: $p=0.003$, $t=4.969$, $df=9$ P6: $p=0.001$, $t=6.049$, $df=9$ P7: $p=0.01$, $t=3.767$, $df=9$
Fig. 36B	Wild-type young Female (WT-YF) (n=7)	Multiple paired t-test	P2: $p=0.12$, $t=2.413$, $df=6$ P3: $p=0.172$, $t=1.555$, $df=6$ P4: $p=0.126$, $t=2.752$, $df=6$ P5: $p=0.035$, $t=4.031$, $df=6$ P6: $p=0.12$, $t=2.744$, $df=6$ P7: $p=0.035$, $t=4.152$, $df=6$
Fig. 36B	RTT-young Female (WT-YF) (n=5)	Multiple paired t-test	P2: $p=0.37$, $t=0.9967$, $df=4$ P3: $p=0.58$, $t=0.5686$, $df=4$ P4: $p=0.19$, $t=1.563$, $df=4$ P5: $p=0.021$, $t=3.667$, $df=4$ P6: $p=0.051$, $t=2.750$, $df=4$ P7: $p=0.030$, $t=3.278$, $df=4$
Fig. 36C	WT-F (n=5)	Multiple paired t-test	P2: $p=0.55$, $t=1.597$, $df=4$ P3: $p=0.55$, $t=1.212$, $df=4$ P4: $p=0.55$, $t=1.504$, $df=4$ P5: $p=0.55$, $t=0.9140$, $df=4$ P6: $p=0.17$, $t=3.218$, $df=4$ P7: $p=0.17$, $t=3.138$, $df=4$
Fig. 36C	RTT-F (n=8)	Multiple paired t-test	P2: $p=0.17$, $t=2.223$, $df=7$ P3: $p=0.17$, $t=2.11$, $df=7$ P4: $p=0.06$, $t=3.405$, $df=7$ P5: $p=0.13$, $t=2.724$, $df=7$ P6: $p=0.17$, $t=1.590$, $df=7$ P7: $p=0.15$, $t=2.505$, $df=7$
Fig. 37A	WT	RM One-Way-ANOVA	F (6,30) = 12.67, $p<0.0001$ P1 vs. P2: $p<0.0001$ P1 vs. P3: $p<0.0001$ P1 vs. P4: $p<0.0001$ P1 vs. P5: $p=0.0002$

			P1 vs. P6: $p<0.0001$ P1 vs. P7: $p<0.0001$
	RTT	RM One-Way-ANOVA	F (6,42) = 15.26, $p<0.0001$ P1 vs. P2: $p<0.0001$ P1 vs. P3: $p<0.0001$ P1 vs. P4: $p<0.0001$ P1 vs. P5: $p<0.0001$ P1 vs. P6: $p<0.0001$ P1 vs. P7: $p<0.0001$
	Normalized-together	RM Two Way-ANOVA	F (6,72) = 1.161, $P=0.3368$
Fig. 37B	WT-YF	RM One-Way ANOVA	F(6,36)=19.36, $P<0.0001$ P1 vs. P2: $p=0.21$ P1 vs. P3: $p=0.0002$ P1 vs. P4: $p<0.0001$ P1 vs. P5: $p<0.0001$ P1 vs. P6: $p<0.0001$ P1 vs. P7: $p<0.0001$
	RTT-YF	RM Two-one ANOVA	F(6,24)=7.514, $p=0.0001$ P1 vs. P2: $p=0.14$ P1 vs. P3: $p=0.017$ P1 vs. P4: $p=0.0014$ P1 vs. P5: $p=0.0008$ P1 vs. P6: $p=0.0003$ P1 vs. P7: $p=0.0004$
	Normalized-together	RM Two-Way ANOVA	F (6,60) = 1.691, $P=0.1389$
Fig. 37C	WT-F	RM One-Way ANOVA	F (6,24)=6.41, $P=0.0004$ P1 vs. P2: $p=0.091$ P1 vs. P3: $p=0.0007$ P1 vs. P4: $p=0.0008$ P1 vs. P5: $p=0.0092$ P1 vs. P6: $p=0.0146$ P1 vs. P7: $p=0.0008$
	RTT-F	RM One-Way ANOVA	F(7,42)=10.52, $P<0.0001$ P1 vs. P2: $p=0.0002$ P1 vs. P3: $p<0.0001$ P1 vs. P4: $p<0.0001$ P1 vs. P5: $p<0.0001$ P1 vs. P6: $p<0.0001$ P1 vs. P7: $p<0.0001$
		RM Two-Way ANOVA	F (6,66) = 1.044, $P=0.4050$
Fig. 38A	Male (WT)	RM Two-Way ANOVA	F(6,30)=9.561, $p=0.0015$
	Male (RTT)	Between baseline and adaptation	F(6,54)=4.199, $p=0.020$
Fig. 38B	Young female (WT-YF)	RM Two-Way ANOVA	F(6,36)=3.847, $p=0.0046$
	Young female (RTT-YF)	Between baseline and adaptation	F(6,24)=1.55, $p=0.03$

Fig. 38C	Old female (WT-F) Old female (RTT-F)	RM Two-Way ANOVA Between baseline and adaptation	$F(6,24)=3.847, p=0.6141$ $F(6,42)=2.119, p=0.0711$
Fig. 39	Male-WT	RM One-Way ANOVA Post-hoc: Tukey's test	$F(3,15)=6.107, p=0.0063$ Baseline vs. 60 min adaptation: $p=0.0023$ Baseline vs. 15 min recovery: $p=0.0324$
	Male-RTT	Mixed effect analysis Multiple comparison	$p=0.0009$ Baseline vs. 60 min adaptation: $p=0.0002$
	Female-young-WT	RM One-Way ANOVA Post-hoc: Tukey's test	$F(3,18)=21.02, p<0.0001$ Baseline vs. 60 min adaptation: $p=0.0002$ Baseline vs. 15 min recovery: $p<0.0001$ Baseline vs. 30 min recovery: $p<0.0001$
	Female-young-RTT	RM One-Way ANOVA Post-hoc: Tukey's test	$F(3,12)=5.007, p=0.0177$ Baseline vs. 60 min adaptation: $p=0.0108$
	Female-old-WT	RM One-Way ANOVA	$F(5,20)=1.461, p=0.24$
	Female-old-RTT	RM One-Way ANOVA	$F(5,35)=1.855, p=0.1276$
Fig.41B	male	RM Two-Way ANOVA Post hoc: Sidak	$F(3,40) = 2.91, p=0.046$ 60 min recovery: $p=0.01$
	Young female	RM Two-Way ANOVA	$F(3,30)=0.5866, p=0.62$
	Old female	RM Two-Way ANOVA	$F(3,55)=0.72, p=0.6$
Fig.41C	male WT (n=6) RTT(n=10)	RM Two-Way ANOVA	$F(3,40) = 2.911, p=0.0272$ 30 min recovery: $p=0.04$
	Young female WT-YF (n=7) WT-RTT (n=5)	RM Two-Way ANOVA Post hoc: Sidak	$F(4,40)=2.718, p=0.043$ 15 min recovery: $p=0.037$ 45 min recovery: $p=0.0289$ 60 min recovery: $p=0.0083$

4 General Discussion

RTT is a neurodevelopment disorder with no cure. Therefore, RTT patients need constant care during their life. Understanding and treating RTT has been delayed by the challenge of repeatedly and noninvasively testing neuronal function in patients throughout the disorder. It is crucial to increase the current knowledge regarding what is missing in the neuronal circuit due to *Mecp2* mutation and find a solution to restore that. The role of MECP2 in regulating the plasticity in development and adulthood has been proved with data from mice models studies (Deng et al., 2010, 2014; Noutel et al., 2011; Na et al., 2013; He et al., 2014; Krishnan et al., 2015, 2017; Tai et al., 2016; Gulmez et al., 2018). We studied two forms of activity-dependent plasticity in *Mecp2* mutant mice to investigate the MECP2 function in sensory processing and neuronal circuits.

We showed the altered long-term plasticity in male Rett model mice and also the inability of the Rett mice model (pre-symptomatic and symptomatic) to retain the long-term adaptation of the central somatosensory circuit. This disruption could be an explanation for the learning and memory dysfunction and cognitive impairment in RTT animals and patients. These data indicate that even before the symptoms start, the brain wiring is likely impaired due to *Mecp2* mutation. However, there was no evidence for peripheral sensory disruption in mutant animals that can potentially affect the parameters of somatosensory evoked responses and cortical plasticity, which is similar to data from visual (LeBlanc et al., 2015) and auditory (Sysoeva et al., 2020; Adcock et al., 2020) studies.

Previously shown that the training of Rett patients should be started at a young age since the environmental enrichment would reduce anxiety and enhance synapse formation in Rett mice if done in P10 (Lonetti et al., 2010). However, our study did not show any evidence

of intact cortical plasticity in younger females, which could be because our mice were at P30 and older.

Our dataset is a valuable first approach to describe the deficit of cortical plasticity *in vivo* in Rett model mice. Still, more studies are needed to be performed before a definitive answer about the role of MECP2 in neuronal wiring and the determination of the location of sensory adaptation in the central nervous system.

It is important to enhance the knowledge about the neuronal abnormalities in RTT, particularly how they function to process the information, potentially a key to designing drugs and applying non-pharmaceutical approaches to improve the patient's quality of life.

4.1 Experimental limitations

In this set of experiments, the duration of cortical recording was about 2.5 hours. During the brain recording sessions, animals were anesthetized with isoflurane. This condition was not ideal for the symptomatic Rett model mice due to the respiratory deficit in this group. However, due to the nature of the piezoelectric, it was not doable to experiment on awake mice. In addition, anesthetizing the experimental animals decreases the higher processing signaling and makes it possible to collect relatively pure sensory inputs. Also, cortical recording from awake animals will increase anxiety which could potentially interfere with sensory signals. It might be possible to record from awake mice during HL stimulation by placing a small vibratory pad, the vibratory stimulation that is not as intensive as piezo, under the paw. However, it may not be possible to induce the same adaptation paradigm with the constant vibration.

HL was stimulated at 100 Hz to induce sensory adaptation. Brain evoked response to HL stimulation was recorded with IOS imaging at 100Hz. To measure the response amplitude with the cortical LFP recording, the sensory-evoked response (SER) was recorded at 10Hz. It

was impossible to increase the stimulation frequency during recording since the SER to a single stimulation lasts for almost 100ms. It is also important to mention that the total duration of SER recording was roughly the same in IOS and LFP, and the result is comparable since we summed that peak amplitude in LFP. Additionally, the data from LFP enabled us to investigate the STA between the stimuli in a train.

4.2 Significance

All the experiments were done *in vivo* (intact brain), meaning neurons received many synapse connections, and the complexity of neuronal wiring was not changed. Therefore, the obtained results reflect the impairment in RTT patient brains more directly.

Most cognitive tests contain locomotion tasks. Since Rett model mice have motor dysfunction, the results from the locomotion test are not very reliable. In contrast, the designed experiments enabled us to probe adaptation properties of the central circuit in Rett animals without locomotor or any complex motor tasks involvement.

The current study was done in both male and female, symptomatic and pre-symptomatic mice. It provided us with information about the role of MECP2 in brain functions, especially regarding the cortical plasticity of homozygous mutant and heterozygous animals. In addition, it gave us information about whether sex or age could affect the level of cortical plasticity.

4.3 Future Direction

Our result showed the observed cortical plasticity in the sensory pathway from HL to the somatosensory cortex is not due to the sensory adaptation at the peripheral afferent ending. Although we found that sensory adaptation occurs at CNS rather than PNS, we were not able

to pinpoint the exact location. Further studies are needed to tackle that down by recording from different parts of HL to the somatosensory pathway.

Additionally, an experiment can be designed *in vitro*. For example, by stimulating the thalamus and recording from the somatosensory cortex (excitatory and inhibitory neurons in L4), we could investigate whether there is any difference in the neuronal response to the adaptation paradigm. For example, if mutant pyramidal cells exhibit excitation at the end of the adaptation period or higher feedback inhibition in the WT cells.

It is also interesting to see whether applying candidate drugs like ketamine or mirtazapine can restore normal brain plasticity. The positive effect of ketamine, NMDA receptor antagonist, has been shown in some of the Rett phenotypes previously (Kron et al., 2012; Patrizi et al., 2016; Ward et al., 2020). Additionally, mirtazapine improves respiratory deficits, cortical atrophy (Bittolo et al., 2016), and normalized parvalbumin levels (Flores Gutiérrez et al., 2020). Working with pre-symptomatic female Rett model mice gives us enough time to apply mirtazapine and study its consequence on plasticity; however, it may not impact the limb to S1 pathway.

5 Bibliography

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