MeCP2 Deficiency is Sufficient to Disrupt Daily Rhythmic Behaviours in Mice

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

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Abstract
Mutations in the X-linked gene encoding Methyl-CpG-binding protein 2 (MECP2) cause the neurodevelopmental disorder Rett syndrome, a common genetic cause of mental retardation in females. Although alterations in performance of MeCP2-deficient mice in specific behavioural tasks have been documented, it remains unclear if, and to what degree, MeCP2 dysfunction affects patterns of periodic behavioural and electroencephalographic activity. To address this, we monitored daily rhythmic patterns of core body temperature, gross motor activity, and cortical delta power from MeCP2-deficient mice and correlated it against regional MeCP2 expression levels. Our results show that normal daily rhythmic behavioural patterning of delta wave activity, body temperature and mobility are disrupted in these mice. Moreover, MeCP2-deficient mice displayed lower average core body temperature and significantly greater body temperature fluctuation than wild-type female mice. Finally, we also found that epileptiform discharge activity in MeCP2-deficient mice is more predominant during times of behavioural activity compared to inactivity.
Acknowledgements

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<th>Description</th>
</tr>
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<tbody>
<tr>
<td>bp</td>
<td>Base pairs</td>
</tr>
<tr>
<td>CWT</td>
<td>Continuous wavelet transform</td>
</tr>
<tr>
<td>c-Ski</td>
<td>c-Ski protein</td>
</tr>
<tr>
<td>CDKL5</td>
<td>Cyclin-dependent kinase-like 5</td>
</tr>
<tr>
<td>dB</td>
<td>Decibel</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribose nucleic acid</td>
</tr>
<tr>
<td>DMNT1</td>
<td>DNA (cytosine-5)-methyltransferase 1</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>ER</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast fourier transformation</td>
</tr>
<tr>
<td>FIR</td>
<td>Finite impulse response</td>
</tr>
<tr>
<td>FOXG1</td>
<td>Forkhead box protein G1</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>HDAC1</td>
<td>Histone deacetylase 1</td>
</tr>
<tr>
<td>HDAC2</td>
<td>Histone deacetylase 2</td>
</tr>
<tr>
<td>Hox-B1</td>
<td>Homeobox protein B1</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>kb</td>
<td>Kilobases</td>
</tr>
<tr>
<td>loxP</td>
<td>Locus of crossover P1</td>
</tr>
<tr>
<td>mPer1</td>
<td>Mammalian period 1</td>
</tr>
<tr>
<td>MBD</td>
<td>Methyl-CpG-binding domain</td>
</tr>
<tr>
<td>MECP2</td>
<td>Methyl-CpG-binding protein 2</td>
</tr>
<tr>
<td>NP40</td>
<td>Nonyl phenoxypolyethoxylethanol</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>NLS</td>
<td>Nuclear localization signal</td>
</tr>
<tr>
<td>N-CoR</td>
<td>Nuclear receptor co-repressor 1</td>
</tr>
<tr>
<td>PMSF</td>
<td>Phenylmethanesulfonylfluoride</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>Tween-20</td>
<td>Polyoxyethylene (20) sorbitan monolaurate</td>
</tr>
<tr>
<td>RIPA</td>
<td>Radioimmunoprecipitation assay</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid-eye movement</td>
</tr>
<tr>
<td>REST</td>
<td>RE-1 silencing transcription factor</td>
</tr>
<tr>
<td>RTT</td>
<td>Rett syndrome</td>
</tr>
<tr>
<td>siRNA</td>
<td>Small interfering ribonucleic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SNpc</td>
<td>Substantia nigra pars compacta</td>
</tr>
<tr>
<td>SCN</td>
<td>Suprachiasmatic nucleus</td>
</tr>
<tr>
<td>TFII B</td>
<td>Transcription factor IIB</td>
</tr>
<tr>
<td>TRD</td>
<td>Transcriptional repression domain</td>
</tr>
<tr>
<td>Tris</td>
<td>Tris(hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>TH</td>
<td>Tyrosine hydroxylase</td>
</tr>
<tr>
<td>VRG</td>
<td>Ventral respiratory group</td>
</tr>
<tr>
<td>XCI</td>
<td>X chromosome inactivation</td>
</tr>
<tr>
<td>ZT</td>
<td>Zeitgeber time</td>
</tr>
<tr>
<td>GABA</td>
<td>( \gamma )-aminobutyric acid</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

1.1. Rett Syndrome (RTT)

1.1.1. Overview of Rett Syndrome

Rett Syndrome, originally characterized by the Austrian paediatrician Andreas Rett, is a neurodevelopmental disorder that is one of the most common genetic causes of mental retardation in females (Rett, 1966). RTT is an X-linked disorder that primarily affects girls at a frequency of approximately 1:10,000 live female births (Laurvick et al., 2006). Greater than 95% of typical presentations of RTT are caused by sporadic mutations in the gene encoding Methyl-CpG-Binding Protein 2 (MECP2) (Amir et al., 1999). The majority of these mutations are missense, nonsense or frameshift mutations but deletion of several nucleotides or even exons has also been observed (Christodoulou et al., 2003; Ravn et al., 2005). Mutations in the genes encoding Cyclin-Dependent Kinase-Like 5 (CDKL5) and Forkhead Box Protein G1 (FOXG1) are also known to cause RTT (Neul et al., 2010).

1.1.2. Methyl-CpG-Binding Protein 2 (MECP2)

The gene, MECP2, is located at Xq28 on the X chromosome and consists of 4 exons which span approximately 76 kilobases (kb). MeCP2 is a member of the methyl-CpG-binding domain (MBD) family of proteins that function to bind to methylated CpG sites (Quaderi et al., 1994). The protein MeCP2 also contains nuclear localization signal (NLS) and transcriptional repression domains (TRD) that allow MeCP2 to associate with
the co-repressor Sin3a which recruits histone deacetylase 1 (HDAC1) and histone
deaetylase 2 (HDAC2). The function of these histone deacetylases is to remove acetyl
groups from nuclear histones which results in a much more compact chromatin structure
that represses genetic transcription of the localized genes (Nan et al., 1998). In addition to
these domains, MeCP2 also contains a carboxy-terminal domain. The function of this
carboxy-terminal domain is not precisely known but it has been shown that disruption of
this region of the protein causes RTT (Neul et al., 2008).

MeCP2 protein is ubiquitously expressed in all tissues but it is relatively more
abundant in the brain. Specifically, MeCP2 is highly expressed in postmigratory neurons
with MeCP2 protein levels being low during embryogenesis and increasing during
postnatal neuronal differentiation (Jung et al., 2003; Shahbazian et al., 2002a). MeCP2
has been reported to be localized in both the cytosol and the nucleus of neuronal cells,
although upon neuronal differentiation MeCP2 is re-localized to mainly the nucleus
(Miyake and Nagai, 2007). Since MeCP2 seems to primarily be expressed in mature
neurons with its levels increasing during neuronal development, it is thought that MeCP2
may be necessary for the maintenance of mature neurons rather than their development.
Consistent with this, it has been observed that MECP2 mutations do not seem to affect
the differentiation or proliferation of neuronal precursors (Chahrour and Zoghbi, 2007).

The most widely accepted view of MeCP2 function is that it acts to repress
genetic transcription through binding of methylated CpG sites and subsequent
recruitment of co-repressors (Figure 1A). As mentioned previously one of these co-
repressors is Sin3a but MeCP2 has also been shown to interact with a variety of other co-
repressors and proteins such as nuclear receptor co-repressor 1 (N-CoR), c-Ski protein (c-
Ski), deoxyribose nucleic acid (DNA) (cytosine-5)-methyltransferase 1 (DMNT1), and transcription factor IIB (TFII B) (Kokura et al., 2001; Kaludov and Wolffe, 2000; Kimura and Shiota, 2003). Additionally, it has also been shown that MeCP2 can promote transcriptional repression of genes through its recruitment to protein complex consisting of RE-1 silencing transcription factor (REST) and CoREST (Lunyak et al., 2002). The precise relevance and consequences of all these protein-protein interactions remains unknown but it seems clear that MeCP2 serves a complex diverse role as a genetic repressor.

Transcriptional profiling studies have been conducted in an attempt to identify the target genes of MeCP2. These gene expression studies done using either human RTT post-mortem tissue or whole brain tissues from mouse models lacking MeCP2 only identified a handful of alterations in gene expression (Colantuoni et al., 2001; Tudor et al., 2002). These results seem to suggest that MeCP2 is not a global transcriptional repressor and that the loss of MeCP2 may only lead to subtle changes in gene expression. From these studies though, some dysregulated genes in the absence of MeCP2 have been identified that may be relevant to the pathophysiology of RTT, those that have received special attention are shown in Table 1.

From these transcriptional profiling studies down-regulated genes were also observed in the absence of MeCP2 suggesting it also functions as a genetic activator (Figure 1B). A study has shown that a majority of activated genes were associated with an enrichment of MeCP2 promoter occupancy in a human neuronal cell line expressing elevated levels of MeCP2 (Yasui et al., 2007). Another study compared gene transcription levels between a mouse model that over-expressed MeCP2 and a mouse
model that lacked MeCP2. This group found distinct target genes that were up-regulated in the presence of excess MeCP2 and down-regulated in the absence of MeCP2, indicating that MeCP2 functioned to activate these target genes (Chahrour et al., 2008). Together, these findings seem to argue against a single role for MeCP2 and that it may serve as both a genetic activator and repressor.

In addition to loss of function mutations in MECP2 causing RTT, gain in MECP2 gene dosage also results in a severe neurological disorder. Duplications of Xq28, the chromosome containing the MECP2 locus, in males have been reported to cause progressive neurodevelopmental phenotypes similar to clinically seen RTT (Friez et al., 2006). Consistent with this, transgenic mouse models over-expressing MeCP2 at twice the normal amount recapitulate the neurological phenotype observed in human males (Collins et al., 2004). These findings suggest that a balance of MECP2 gene expression is essential for normal healthy development and that skewing MECP2 in either direction will result in similarly abnormal phenotypes.

1.1.3. Typical RTT Progression

Girls presenting with classical RTT appear to develop normally for the first 6-18 months of age before experiencing a period of psychomotor regression (Figure 2). Affected girls start by experiencing a deceleration in head growth rates which eventually leads to microcephaly (Reiss et al., 1993). Additionally, the neurons of RTT brains have also been found to be smaller and more densely packed and show reduced dendritic numbers (Belichenko et al., 1994). Coinciding with the acquired microcephaly, patients start to show general growth retardation, weight loss, and hypotonia (Chahrour and
Zoghbi, 2007). With the onset of the regression stage, acquired speech and motor skills are lost and affected children start to develop hand wringing or washing stereotypies as well as ataxia (Hagberg et al., 1983). These patients then start to display many autistic features such as social withdrawal, increased irritability, unresponsiveness to social cues, increased anxiety-like behaviour, and cognitive disability (Kaufmann et al., 2011).

As the child continues to progress, widespread autonomic dysfunction and respiratory abnormalities begin to present. Breathing abnormalities such as hyperventilation during wakefulness, breath-holding, aerophagia, forced expulsion of air, and apnea are all commonly found in RTT girls (Weese-Mayer et al., 2006). RTT patients also suffer from abnormal sleeping patterns and disrupted daily rhythms. It has been found that girls with RTT display reduced amounts of overall and rapid-eye-movement (REM) stage sleep (Glaze et al., 1987). Additionally, it has been observed that RTT patients also display increased day-time sleep, decreased night-time sleep, increased night-time waking, delayed sleep onset, and early waking periods than age-matched controls (Piazza et al., 1990; Ellaway et al., 2001). Thermoregulatory deficits are also observed in RTT patients and they often display hypothermia in their extremities (Naidu et al., 1987). Cardiac abnormalities are also observed in these patients such as tachycardia, prolonged QT intervals, and sinus bradycardia. Finally, autonomic dysfunction in these girls leads them to have multiple gastrointestinal problems such as gastrointestinal dysmotility, chewing and swallowing difficulties, and biliary tract disorders (Motil et al., 2012).

Another feature of RTT is the occurrence of epileptic seizures. In most cases, the onset of these seizures is at around 2 years of age and they increase in severity until 7-12
years of age (Cooper et al., 1998; Glaze et al., 2010; Jian et al., 2007). There is a variety of seizure types that affect Rett syndrome patients and they can often co-exist in the same patient, the most important of which are tonic, absence, atypical absence, atonic, and partial complex seizures (Pardal-Fernández et al., 2004; Niedermeyer et al., 1986). RTT patients present with abnormal electroencephalogram (EEG) recordings that usually include epileptiform findings. Not all of these epileptiform events present with behavioural components, suggesting seizures tend to be under-recognized by the parents of the RTT girls. Additionally, many events parents claim as seizures tend to be ‘non-epileptic’ as there is no underlying abnormal EEG activity correlating with those episodes (Glaze et al., 1998).

Finally, RTT patients tend to experience a late motor deterioration stage. This stage is characterized by scoliosis, worsening dystonia, rigidity, and further loses in the ability of these patients to walk. Patients often develop Parkinsonian features such as hypomimia, freezing, and akinesia (Hagberg et al., 2005; Roze et al., 2007). The overall longevity of RTT women is certainly diminished compared to healthy women with affected individuals having a yearly death rate of 1-2%, with 25% of all these deaths characterized as sudden and unexpected (Kerr et al., 1997). Still, despite the multitude and severity of the symptoms these girls experience, some RTT patients have lived past 60 years of age (Kirby et al., 2010).

1.1.4. Atypical Presentations of RTT

A variety of specifically defined variant forms of RTT that differ from the classical presentation of RTT have been observed. These variants can be either milder or
more severe in comparison to typical RTT. Three more common distinct variant forms of RTT are the preserved speech variant, the congenital variant, and the early seizure variant (Neul et al., 2010). The preserved speech variant is mainly caused by mutations in MECP2, whereas mutations in CDKL5 have been associated with the early seizure variant and mutations in FOXG1 have been found in the congenital variant (Bahi-Buisson et al., 2008; Ariani et al., 2008). The preserved speech variant is a milder variant with the regression stage occurring later in development, between 1 and 3 years of age (Zappella, 1992). Patients with this variant tend to have less reduction in hand and motor skills and recover some language skills after regression. These patients also tend to have milder intellectual deficits and rarer occurrences of epilepsy and autonomic dysfunctions. Girls with the early seizure RTT variant are characterized by the onset of seizures before 5 months of age (Hanefeld, 1985). These girls experience infantile spasms and refractory myoclonic epilepsy but tend to not display many typical RTT features. The congenital variant of RTT presents with grossly abnormal initial development with severe psychomotor delay with the infant never acquiring the ability to walk (Rolando, 1985). In these cases microencephaly presents before 4 months of age and the regression stage begins to occur before 5 months of age.

1.1.5. Phenotypic Variance in RTT

Both classical and atypical variant phenotypes of RTT can vary significantly in severity and onset between different patients and even in the same patient over time. The main source of phenotypic severity in females with MECP2 mutations is the patterning of X chromosome inactivation (XCI) in the patient. As MECP2 is located on the X
chromosome and in females only one of the two X chromosomes is active in a given cell, there is a random chance of the cell either expressing the wild-type MECP2 allele or the mutant MECP2 allele. Therefore a female will typically be mosaic, expressing the wild-type allele in half her cells while the mutant allele in the other half. Occasionally though, there might be preferential skewing towards one of the alleles which results in either a relatively more severe or milder presentation of RTT, this is best illustrated by monozygotic twins with RTT that manifest two very different phenotypes (Dragich et al., 2000). There is also evidence of somatic mosaicism in females with MECP2 mutations, which may also contribute to the phenotypic variability observed among RTT patients (Bourdon et al., 2001).

As mentioned previously, RTT is primarily only observed in females with very few cases being present in males. This is due to the lack of a second X-chromosome in the males that results in a homozygous genotype for the mutated MECP2 gene. Whereas the females have a mosaic pattern of cells expressing functional and non-functional MeCP2, none of the male cells express functional MeCP2 and this typically results in severe neonatal encephalopathy and death in utero or within the first year of life (Schanen et al., 1998). Yet, there are some cases of males with RTT syndrome. Males with Klinefelter syndrome, a disorder resulting in a 47, XXY karyotype, and a MECP2 mutation on one of the X chromosomes will present with a classic RTT phenotype (Schwartzman et al., 2001). Additionally, males with a normal karyotype (46, XY) and MECP2 mutations have been found to present with typical RTT phenotypes, presumably due to genetic modifiers that suppress the infantile lethality typically associated with homozygous loss of function MECP2 mutations (Budden et al., 2005, Dayer et al., 2007).
Figure 1

A

B
Figure 1. Function of MeCP2

Panel A: MeCP2 can act as a transcriptional repressor. The methyl binding domain (MBD) of the MeCP2 protein binds to methylated-CpG islands upstream of the transcriptional start site of a target gene of MeCP2. This in turn causes the recruitment of co-repressors such as Sin3a and histone deacetylases (HDACs) which cause local chromatic compaction and transcriptional down-regulation. Panel B: MeCP2 may also act as a transcriptional activator by recruitment of transcriptional co-activators which cause the MeCP2 target gene to be transcriptional up-regulated.

Modified figure from: Samaco and Neul, 2011.
Figure 2. Typical RTT Symptom Progression Timeline

In cases of typical RTT, a healthy baby girl begins developing normally and reaches the appropriate milestones, but at around 12-18 months of age experiences developmental stagnation and a period of rapid regression. During this rapid regression period, previously acquired skills such as speech, locomotion, and the ability to social interact are lost and the girl begins to develop hand stereotypies, severe cognitive impairments, motor abnormalities, seizures, abnormal anxiety-like behaviour, as well as widespread autonomic dysfunction. In the late stages of disease progression, motor function further deteriorates and RTT patients often present with Parkinsonian features.

Modified figure from: Chahrour and Zoghbi, 2007.
Table 1. Summary Table of Extensively Studied Target Genes of MeCP2.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bdnf</td>
<td>neuronal development and survival</td>
<td>Chen et al., 2003; Martinowich et al., 2003</td>
</tr>
<tr>
<td>xHairy2a</td>
<td>neuronal repressor</td>
<td>Stancheva et al., 2003</td>
</tr>
<tr>
<td>DLX5/Dlx5</td>
<td>neuronal transcription factor</td>
<td>Horike et al., 2005</td>
</tr>
<tr>
<td>Sgk1</td>
<td>hormone signaling</td>
<td>Nuber et al., 2005</td>
</tr>
<tr>
<td>Fkbp5</td>
<td>hormone signaling</td>
<td>Nuber et al., 2005</td>
</tr>
<tr>
<td>Uqrc1</td>
<td>mitochondrial respiratory chain</td>
<td>Kriaucionis et al., 2006</td>
</tr>
<tr>
<td>ID1-3/Id1-3</td>
<td>neuronal transcription factor</td>
<td>Peddada et al., 2006</td>
</tr>
<tr>
<td>FXYD1/Fxyd1</td>
<td>ion channel regulator</td>
<td>Deng et al., 2007</td>
</tr>
<tr>
<td>UBE3A</td>
<td>ubiquitin ligase</td>
<td>Samaco et al., 2005</td>
</tr>
<tr>
<td>GABRB3</td>
<td>GABA-A receptor</td>
<td>Samaco et al., 2005</td>
</tr>
</tbody>
</table>
1.2. Mouse Models of Rett Syndrome

1.2.1. Different Mouse Models of RTT

To assist in uncovering the molecular changes underlying RTT and to allow for preclinical translational studies many mouse models of RTT have been developed. These mouse models either lack MeCP2 or express a relevant mutant form of MeCP2 and tend to recapitulate many of the behavioural and neurological deficits observed in clinical RTT.

One of the first mouse models of RTT developed, referred to as the $Mecp2^{tm1.1Bird}$ model, was a mouse in which exons 3 and 4 of $Mecp2$ were excised out resulting in the deletion of all but the amino-terminal eight amino acids of MeCP2 (Guy et al., 2001). Briefly, this mouse model was generated by transfecting embryonic stem cells with a targeting vector that replaced exons 3 and 4 of the $Mecp2$ gene with the same exons flanked by locus of crossover P1 ($loxP$) sites allowing the generation of $Mecp2^{lox/lox}$ female mice. These $Mecp2^{lox/lox}$ female mice were then crossed with deleter mice which expressed Cre recombinase ubiquitously. The resulting offspring had the $loxP$-flanked exons 3 and 4 excised by the ubiquitously expressed Cre generating $Mecp2^{-/-}$ female mice and $Mecp2^{-y}$ male mice which showed symptoms that mimicked RTT. At around the same time as the $Mecp2^{tm1.1Bird}$ model was developed another group also developed a mouse model which showed similarities to RTT. This mouse model, referred to as the $Mecp2^{Jae}$ model, was generated in a similar fashion to the other one except that only exon 3 was excised (Chen et al., 2001). Another similar mouse model of RTT that was generated using the same method is the $Mecp2^{tm1Tam}$ mouse model. $Mecp2^{tm1Tam}$ expresses
a mutant form of MeCP2 that is missing the MBD region ubiquitously but otherwise is intact (Pelka et al., 2006).

Other newer mouse models have been developed that express clinically relevant mutant forms of the MeCP2 protein. One model, which expresses a truncated form of MeCP2 and is referred to as $Mecp^{208}$, has had a premature stop codon inserted after codon 308 so as to resemble clinically seen truncation mutations (Shahbazian et al., 2002b). This truncated protein retains the MBD, TRD, and NLS regions of the protein but the C-terminal third of the coding sequence has been removed. Another mouse model of RTT is the $Mecp^{A140V}$ model, which has a missense mutation changing the 140th amino acid in MeCP2 from an Alanine to a Valine (Jentarra et al., 2010). This mutation occurs within the MBD region of the MeCP2 protein but does not seem to affect the ability of the mutant MeCP2 protein to associate with methylated CpG sites. The $MECP2$ A140V mutation contributes to approximately 0.6% of clinical RTT cases and these patients present with a very distinct atypical variant that more closely resembles X-linked mental retardation than classic RTT. A third mouse model displaying a clinically seen mutant form of the MeCP2 protein has also been developed by substituting the 158th amino acid, Threonine, with Alanine which is located at the C terminus of the MBD region (Goffin et al., 2011). This $Mecp^{T158A}$ mouse model was developed as T158M is one of the most common $MECP2$ mutations, comprising 10% of all cases. Although the clinical mutation is T158M, the T158A mutation was easier to implement in a mouse model and this mutation causes the same clinical RTT manifestation as the T158M mutation.
Finally, there has been a mouse model of RTT developed that lacks MeCP2 but a functional MeCP2 protein may be induced by injection of the estrogen receptor (ER) antagonist, Tamoxifen. This mouse model, known as \textit{Mecp}^{2\text{tm}2\text{Bird}} \text{, has had a } \textit{loxp}\text{-flanked stop cassette spliced into intron 2 of the Mecp2 gene (between exon 2 and 3) with its own splice acceptor and donor regions (Guy et al., 2007). When transcribed, the resulting transcript stops after exon 2 resulting in a non-functional MeCP2 transcript and a lack of functional MeCP2 protein. These mice also express Cre recombinase but fused to a modified estrogen receptor (\textit{cre-ER}) which causes it to remain in the cytoplasm until exposed to Tamoxifen. When Tamoxifen is administered, it binds to the ER associated with Cre allowing it to be translocated to the nucleus whereby it excises the \textit{loxp}\text{-flanked stop cassette which causes a functional full length MeCP2 transcript to be produced and the restoration of MeCP2 protein expression. This mouse model allows for selective re-activation of MeCP2 at specific time points in the mouse’s development to assess whether lacking MeCP2 during developmental stages produces non-reversible detrimental effects. An important note about this mouse model though, is that occasionally intact MeCP2 transcripts are transcribed as the splicing regions of the stop cassette are sometimes skipped over during transcription. This phenomenon results in \textit{Mecp}^{2\text{tm}2\text{Bird}} \text{ mice not being complete nulls and expressing slightly more functional MeCP2 protein than } \textit{Mecp}^{2\text{tm}1.1\text{Bird}} \text{ mice.}
1.2.2. General Phenotypes of RTT Mouse Models

All of the mouse models of RTT have similar developmental phases as what is seen clinically in RTT patients. The mouse models all show an early developmental phase where there is normal progression with no obvious deficits before experiencing a period of regression and worsening symptoms culminating in premature death. The time of onset and severity of symptoms varies greatly between both the mouse model used and gender of mouse. The phenotypes of the various different mouse models are summarized in Table 2.

In the \textit{Mecp2}^{tm1.1Bird} mouse model, both male and female mice develop similar gross abnormalities but with sexually distinct time courses (Guy et al., 2001). These mice often develop a stiff, uncoordinated gait and perform reduced amounts of spontaneous movements. Additionally, these mice also present with hind limb clasping behaviour, irregular breathing patterns, tremors, and disheveled fur. Jaw misalignment and uneven wearing of teeth were also commonly observed in this model. The overall body weight of these mice was dependent on the strain of mouse used, \textit{Mecp2}^{tm1.1Bird} mice reared on a C57BL/6 genetic background gave rise to underweight animals whereas those reared on a 129 genetic background gave rise to heavier animals compared to wild-type siblings. In the males, these abnormalities begin to develop at around 3-4 weeks postnatal and continue to progress until around 8 weeks postnatal at which point the male mice experience rapid weight loss and death. In contrast, the females remain asymptomatic for much longer, only developing symptoms at around 4 months postnatal and they can live as long as wild-type mice. Generally, the symptomatic severity of female mice is less
overall than the males and often the females do not present with all of the aforementioned abnormalities.

The general phenotype of the $Mecp2^{lae}$ mouse model is very similar to that of the $Mecp2^{tm1.1Bird}$ model. $Mecp2^{lae}$ male mice begin to develop symptoms at around 5 weeks of age showing abnormal behaviour such as body trembling, abnormal gait, and laboured breathing. The average body weight of these male mutants is still in debate as there are conflicting results with one group claiming an overweight phenotype whereas another group observes an underweight phenotype (Chen et al., 2001; Stearns et al., 2007). At around 8 weeks these mice experience extreme physical deterioration developing a hypoactive phenotype and weight loss with death at approximately 10 weeks of age. Much like in the $Mecp2^{tm1.1Bird}$ model, female $Mecp2^{lae}$ mice remain asymptomatic until around 4 months postnatal before developing abnormalities similar to what is seen in the male mice. Premature deaths in $Mecp2^{lae}$ female mice have not been observed. The other MeCP2-null mouse model, $Mecp2^{tm1Tam}$, also develops the same general phenotype as the $Mecp2^{tm1.1Bird}$ and $Mecp2^{lae}$ models. The male mice start to present with unusual gait, hind limb clasping, disheveled fur, labored breathing, tremors and reduced body weight at 5 weeks postnatal and have been reported to survive up to 20 weeks of age (Pelka et al., 2006). Female $Mecp2^{tm1Tam}$ mice are asymptomatic until 3-4 months of age with no reports of premature death. Overall, these three MeCP2-null mouse models ($Mecp2^{tm1.1Bird}$, $Mecp2^{lae}$, and $Mecp2^{tm1Tam}$) display similar phenotypic impairments and developmental time courses with the $Mecp2^{tm1.1Bird}$ model being the most severely affected.
The *Mecp2*<sup>308</sup> mouse model, which expresses a truncated mutant form of MeCP2, has a distinct phenotype from the previously mentioned MeCP2-null mouse models. The mutant male mice of this model do not exhibit any abnormalities until 6 weeks of age, when tremors can be seen in the mice when suspended by their tail (Shahbazian et al., 2002b). These tremors progress until they become apparent by visual observation alone at around 4 months of age. These mice also tend to develop kyphosis (abnormal curving of the spine resulting in a hunchback appearance), and have disheveled, oily fur as compared to wild-type mice by 8 months of age. Additionally, these mice are reported to display ataxia, breathing abnormalities, and fore limb stereotypies in their home cage setting (Moretti et al., 2005). The average body weight of these mice is consistent with what is observed in wild-type mice and they are fertile as opposed to the MeCP2-null models. The majority of *Mecp2*<sup>308</sup> male mice live past one year of age with only 10% dying abruptly after 10 months. Female *Mecp2*<sup>308</sup> mice display milder and more variable features starting at 1 year of age.

*Mecp2*<sup>T158A</sup>, a mouse model expressing a mutated full length MeCP2 protein product, develops a phenotype similar to the MeCP2-null mouse strains. *Mecp2*<sup>T158A</sup> males begin to display symptoms at 4 weeks, the same time as *Mecp2*<sup>tm1.1Bird</sup> male mice, but generally live a little longer than the *Mecp2*<sup>tm1.1Bird</sup> model, with 50% dying by 16 weeks of age (Goffin et al., 2011). *Mecp2*<sup>T158A</sup> female mice, similar to the other models, develop symptoms much later in development, at around 4-5 months of age, and do not show any signs of early lethality. Overall, the *Mecp2*<sup>T158A</sup> mouse model manifests RTT-like phenotypes but at a lesser extent than the MeCP2-null models. In contrast to all of these other mouse models, the *Mecp2*<sup>A140V</sup> model does not develop an abnormal
phenotype. Male mice had an apparent normal lifespan and normal weight gain patterns with no obvious tremors, seizures, breathing difficulties or kyphosis (Jentarra et al., 2010).

1.2.3. Motor Impairments in RTT Mouse Models

Motor impairments are a typical feature of RTT and there has been a lot of research into characterizing the extent of motor impairment in the mouse models of RTT. In the Mecp$^{tm1.1Bird}_2$ model it has been reported that both male and female mice have a reduced latency to fall off a rotating rod and show decreased overall activity in the open field arena behavioural test (Guy et al., 2001; Santos et al., 2007; Jugloff et al., 2008). Mecp$^{tm1.1Bird}_2$ male mice have also been reported to display reduced grip strength, as measured by the time period until falling when suspended by the forepaws on a taut wire (Pratte et al., 2011). In contrast to these results thought, it has also been observed that Mecp$^{tm1.1Bird}_2$ male and female mice spend a longer time suspending from a wire compared to age-matched wild-type controls (Santos et al., 2007). Similar results to what is seen in Mecp$^{tm1.1Bird}_2$ mice have been found in the Mecp$^{Jae}_2$ mice. Both male and female Mecp$^{Jae}_2$ mice show reduced exploratory and ambulatory movement in the open field arena and shorter times spent on the rotating rod without falling (Stearns et al., 2007). Grip strength was also reported to be significantly lower in these mice compared to wild-type controls. Mecp$^{tm1Tam}_2$ male and female mice, similar to the other MeCP2-null models, have also been reported to have reduced latency to falling off a rotating rod, indicating impaired motor co-ordination (Pelka et al., 2006; Kondo et al., 2008). Extensive characterization of motor deficits has been conducted in Mecp$^{308}_2$ male mice,
revealing shorter latencies to drop in wire suspension, wooden dowel, and vertical pole behavioural tests. Additionally, slightly reduced locomotor activity in the open field has been reported but no significant deficits in rotating rod performance have been noted in these mice (Shahbazian et al., 2002b; Moretti et al., 2005). Mild abnormalities in the patterning of motor activity in the MeCP2<sup>308</sup> male mice has also been identified, namely, there is an observed decrease in activity in the dark phase and hyperactivity in the light phase of a 12:12 hour diurnal cycle. Finally, decreased home-cage activity amounts and impaired rotating rod performance has also been reported in MeCP2<sup>T158A</sup> male and female mice (Goffin et al., 2011).

The widespread motor deficits resulting from a lack of MeCP2 is not very surprising as MeCP2 is known to be heavily expressed in motor cortical and cerebellar regions of the brain, both of which are implicated in motor co-ordination and performance (Mullaney et al., 2004). In the motor cortex of MeCP2-null mice it has been observed that there is reduced dendritic spine density and size as well as abnormal axonal organization, suggesting disrupted neuronal networks (Belichenko et al., 2009). Similarly, layer 2/3 pyramidal cells of the motor cortex have been observed to possess reduced soma size, dendritic complexity and length (Robinson et al., 2012). Additionally, impaired dopaminergic function has been reported in MeCP2-null mice suggesting nigrostriatal deficits may play a role in genesis of parkinsonian-like motor dysfunction in RTT. Reduced catecholamine metabolites and synthesis have been reported in MeCP2-null mice (Samaco et al., 2009). Reduced levels of dopamine amount, release, and dopaminergic currents have also been observed in the dopaminergic neurons of the substantia nigra (Gantz et al., 2011; Panayotis et al., 2010). It has also been shown that
there are reduced tyrosine hydroxylase (TH) expressing neurons, an enzyme necessary in
the pathway of generating dopamine, in the substantia nigra pars compacta (SNpc) and
caudate-putamen brain regions of MeCP2-null mice (Panayotis et al., 2010).

1.2.4. Abnormal Anxiety-Like Behaviour in RTT Mouse Models

Differences in anxiety profiles have been noted in Meq2\textsubscript{Jae} mice but whether it is
heightened or reduced is unclear. Mutant males and females were found to spend more
time than controls in the open arms of both the zero and elevated plus-mazes, indicative
of lessened anxiety, but they also displayed greater amounts of time freezing on the zero
maze, suggesting heightened anxiety (Stearns et al., 2007). In contrast, anxiety profiles
have not been found to be altered in either sex of Meq2\textsubscript{m1.1Bird} mice as tested using both
the open field arena and elevated plus-maze behavioural tests (Santos et al., 2007). Increased anxiety-like behaviour has been observed in the male mice of the Meq2\textsuperscript{308}
model. These mice display reductions in exploratory behaviour in an open field setting,
spend reduced time in the open arms of an elevated plus-maze, and spend more time in
the dark compartment during a light-dark placement preference test (Shahbazian et al.,
2002\textsubscript{b}; McGill et al., 2006). Additionally, these mice also showed fewer transitions
between the light and dark compartments in the placement preference test, indicative of
reduced risk taking behaviour (McGill et al., 2006). In contrast, reduced anxiety-like
behaviour has been observed in the Meq2\textsuperscript{m1Tam} and Meq2\textsuperscript{T158A} mouse models, as they
spent increased amounts of time in the open arm of an elevated plus-maze (Pelka et al.,
2006; Goffin et al., 2011).
Despite the ambiguity seen in anxiety-like behaviours in these mouse models, more specialized targeted MeCP2 knockout models have reinforced the finding of increased anxiety-like behaviour. Reducing MeCP2 specifically from the basolateral amygdala (BLA), a structure known to be crucial to anxiety behaviour expression, has been found to result in heightened anxiety-like behaviour in mice (Adachi et al., 2009). Similarly, forebrain-specific knockout of MeCP2 in mice resulted in an increased anxiety phenotype (Gemelli et al., 2006). The hippocampus is also known to be involved in the expression of anxiety-like behaviours (Barkus et al. 2010, McHugh et al. 2004). A possible link between increased anxiety and MeCP2-deficiency, is that serotonergic innervation in the hippocampus is disrupted in MeCP2-deficient mice. Serotonin (5-hydroxytryptamine) and its main metabolite (5-hydroxyindoleacetic acid) have been found to be markedly reduced in the hippocampus of MeCP2-deficient mice and serotonin transporter knockout mice display similar heightened anxiety-like behaviour to MeCP2-deficient mice (Isoda et al. 2010, Ren-Patterson et al. 2005).

1.2.5. Autonomic Dysfunction in RTT Mouse Models

Current research has focused heavily on characterizing the breathing impairments of these mouse models. Yet, there has been very little research on the cardiac, thermoregulatory, and daily rhythmic behavioural systems of these mice. Additionally, absolutely no studies on the gastrointestinal tract of these mice have been undertaken despite there being prevalent gastrointestinal problems in RTT patients. Abnormal breathing profiles have been visually observed in all of the previously mentioned mouse models but the only in-depth look at the breathing irregularities was in MeCP2$^{tm1.Bird}$
mice. Irregular ragged breathing patterns of fast and slow respiratory frequencies, periodic breathing, as well as apneas have been reported in the male \textit{Mecp2}^{\text{tm1.1Bird}} mice (Viemari et al., 2005; Abdala et al., 2010). Additionally, respiratory depression following hyperventilation in hypoxic conditions has been observed in female \textit{Mecp2}^{\text{tm1.1Bird}} mice (Bissonnette and Knopp, 2006). These results coupled with reports that MeCP2-deficient mice have larger tidal and lung volumes than wild-type mice could suggest that knockout female mice suffer from hypocapnia, a diminished concentration of carbon dioxide in the blood (Ricceri et al., 2008; Bissonnette and Knopp, 2006).

The breathing irregularities seen in the mouse models of RTT are thought to be primarily caused by impairments in the normal activity of the expiratory neurons in the ventral respiratory group (VRG) region of the brain. \textit{In vitro} recordings from the VRG of \textit{Mecp2}^{\text{tm1.1Bird}} revealed highly irregular cyclic population bursts in contrast to the highly regular rhythmic activity seen in wild-type mice (Viemeri et al., 2005). This was ameliorated by administration of norepinephrine (NE) which has been noted to be reduced in medullary neurons of MeCP2-null mice (Viemari et al., 2005). The irregular VRG network activity is thought to arise from an imbalance in the excitation and inhibition inputs to this system and in line with this, enhanced excitation in the brainstem has been observed in \textit{Mecp2}^{\text{tm1.1Bird}} male mice (Medrihan et al., 2008).

Cardiac arrhythmias have been observed in \textit{Mecp2}^{\text{tm1.1Bird}} male and female mice, specifically these mice have prolonged corrected QT intervals and are susceptible to sustained ventricular tachycardia (McCauley et al., 2011). These abnormalities are thought to arise from an increased persistent sodium current in the cardiomyocytes of these mice, which has been noted in other models of long QT. The precise mechanism
leading to this increased sodium current and the cardiac arrhythmias are unknown, but neurological dysfunction has been hypothesized to play a role. Repetitive seizures, which are present in RTT patients and mice, have been shown to remodel the distribution of potassium and sodium channels within the heart, leading to QT elongation and arrhythmias (Bealer et al., 2010).

Little research into thermoregulatory and daily rhythmic behaviours has been conducted on RTT mouse models despite RTT girls showing disrupted daily patterns and abnormal temperature profiles. The extent of thermoregulatory characterization in RTT mouse models is one study that observed lower subcutaneous temperatures in $Mecp2^{tm1.1Bird}$ male mice compared to wild-type controls (Ward et al., 2011). This deficit in thermoregulation most likely arises from abnormalities in the hypothalamus, a structure crucial to controlling body temperature. In fact, MeCP2 deficiency has been shown to cause abnormal gene expression specifically in the hypothalamus and removing MeCP2 solely from Homeobox protein B1 (Hox-B1) derived tissues, which excludes the hypothalamus, was insufficient to cause reduced body temperatures in $Mecp2^{tm1.1Bird}$ male mice (Ben-Shachar et al., 2009; Ward et al., 2011).

Finally, the only research into daily rhythmic behaviour patterns in RTT mouse models has been one study that observed relative hypoactivity in the dark phase and hyperactivity in the light phase of $Mecp2^{308}$ male mice compared to wild-type mice during a 12:12 light:dark cycle (Moretti et al., 2005). The lack of research into daily behavioural patterning of MeCP2-deficient mice is surprising as there is considerable evidence that lacking MeCP2 would manifest irregular rhythmic behavioural cycles. Recent studies have found $Mecp2$ mRNA to be a direct target of the microRNA miR-132.
Within the neurons of the suprachiasmatic nucleus (SCN), a brain region responsible for controlling circadian rhythms, miR-132 expression is robustly induced by light stimulation (Cheng et al., 2007), and miR-132 negatively regulates MeCP2 protein levels in these neurons. This regulation of MeCP2 expression is one component of the system regulating the expression of Period genes and thus contributes to clock entrainment. Furthermore, disruption of clock gene regulation in SCN is sufficient to alter daily cortical delta periodicity and power (Franken and Dijk, 2009).

1.2.6. RTT Mouse Models Display Epileptiform Discharges

Despite epileptic seizures being one of the most severe co-morbidities in RTT patients, very little research has been conducted looking at seizure phenotypes in the RTT mouse models. Two studies have found epileptiform discharge events, indicative of epileptic seizures, in the MeCP2\textsuperscript{tm1.1Bird} and MeCP2\textsuperscript{308} mouse models. Myoclonic jerks coupled with high-amplitude bilateral cortical spike and wave EEG discharges have been observed in the MeCP2\textsuperscript{308} male mouse model. Rhythmic spike and wave EEG discharges have also been observed in MeCP2\textsuperscript{tm1.1Bird} female mice (Shahbazian et al., 2002\textit{b}; D’Cruz et al., 2010). These discharges seem to be absence-like in nature as they are severely attenuated with the administration of ethosuximide, a widely used anti-absence drug, and are accompanied by behavioural arrest (D’Cruz et al., 2010). It is thought that these rhythmic spike and wave discharges arise through thalamo-cortical network dysfunction, as these networks have been implicated in other rodent models of absence seizures (Crunelli and Leresche, 2002; McCormick and Contreras, 2001). The precise mechanistic
deficit underlying these epileptic discharges is unknown, but MeCP2 has been shown to be crucial for normal γ-aminobutyric acid (GABA) signalling and circuitries (Chao et al., 2010; Zhang et al., 2010), and dysfunctions in GABA signalling are known to cause the genesis of absence-like epileptic seizures in rodent models (Banerjee et al., 1993; Fariello and Golden, 1987; Cope et al., 2009).

1.2.7. X-Chromosome Inactivation and Phenotypic Variability in RTT Mouse Models

As seen clinically in RTT patients, there is also a wide variety in phenotypic severity observed in the female mice of RTT mouse models. The main contributor to this phenotypic severity is X-Chromosome Inactivation (XCI) patterning in the specific tissues of the mouse. Studies have shown that the patterning of XCI correlates with the probability of a mouse developing certain RTT phenotypic characteristics. The more the XCI patterns favoured the expression of the wild-type allele in Mecp2 female mice the smaller the odds of the mouse exhibiting tremors, stereotypic forepaw movements, disheveled fur, and periocular lesions (Young and Zoghbi, 2004). Additionally, this study noted that the XCI patterns were non-random and unbalanced, favouring the expression of the wild-type allele over the mutant allele. They also showed selective survival of the cells carrying the wild-type allele as opposed to those possessing the MeCP2 allele, indicating that skewed cell survival might explain the unbalanced XCI patterns observed in the female mice.

XCI skewing is not the only influencer of MeCP2 expression levels, as studies have shown that MeCP2 can be influenced by micro-RNA regulation of the Mecp2 transcript (Klein et al., 2007; Alvarez-Saavedra et al., 2011) and through post-
translational MeCP2 protein modifications (Zhou et al., 2006; Rexach et al., 2010). Additionally, it has also been shown that wild-type \textit{Mecp2} allele expression can be influenced by surrounding \textit{Mecp2}^{tm1.1Bird} expressing cells (Braunschweig et al., 2004). Through non-cell autonomous mechanisms, surrounding mutant allele expressing cells negatively influenced neighbouring wild-type allele expressing cells causing them to express significantly lower amounts of MeCP2 protein as compared to complete wild-type mice. Together, these findings indicate that MeCP2 expression in the females is variable, non-random, and under complex control. While there is some evidence linking the levels of MeCP2 to phenotypic severity, the current studies examine only superficial behaviours against a select few neuronal cell populations.

1.2.8. The Better Mouse Model: Male Versus Female

Despite RTT predominately affecting girls, the vast majority of mouse studies have focused on the phenotypes of hemizygous male mice. The main reason behind this discrepancy is the large variance in phenotypic presentation of the heterozygous females due to XCI (Ricceri et al., 2008). This variability in phenotypes provides obstacles to investigating the precise role of MeCP2 on the development of RTT-like symptoms. As such, studying male mice is preferred as they typically present with a more consistent phenotype across subjects. An additional consideration that causes researchers to prefer male mice is that they tend to develop more severe symptoms and at a significantly quicker pace than the female mice. The most commonly used male knockout mouse models, \textit{Mecp2}^{tm1.1Bird} and \textit{Mecp2}^{Jae}, start to display an overt phenotype at approximately 30-40 days of age and die between 60-90 days of age (Guy et al., 2001; Chen et al.,
Whereas, the female mice of these models do not start developing RTT-like symptoms until 3-4 months of age and can live full length lives. These obstacles make it significantly simpler and less arduous to study the male model rather than the female model. Although studying the female model presents problems, the female mice are more clinically relevant and as such there has been a growing shift in interest towards examining the phenotypic impairments in the female mice of the RTT mouse models.
Table 2. Summary Table of Rett Mouse Model Phenotypes

<table>
<thead>
<tr>
<th>Constitutive Knock-Out</th>
<th>Clinical Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mecp2&lt;sup&gt;tm1.1Bird&lt;/sup&gt;</strong></td>
<td><strong>Mecp2&lt;sup&gt;lde&lt;/sup&gt;</strong></td>
</tr>
<tr>
<td><strong>Symptom Onset</strong></td>
<td>Male: 3 weeks of age</td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td>Male: 8 weeks of age Female: No premature death</td>
</tr>
<tr>
<td><strong>General Phenotype</strong></td>
<td>Male/Female: Stiff gait, reduced movement, hind limb clasp, tremors, and disheveled fur, C57BL/6 mice underweight, 129 mice overweight</td>
</tr>
<tr>
<td><strong>Motor Impairments</strong></td>
<td>Male/Female: Lower time on rotarod, decreased open field activity, mixed results on grip strength</td>
</tr>
<tr>
<td><strong>Anxiety-like Behaviour</strong></td>
<td>Male/Female: No abnormalities as tested using open field or elevated plus-maze tests</td>
</tr>
<tr>
<td><strong>Autonomic Dysfunction</strong></td>
<td>Male: Periodic breathing, irregular ragged breathing, apneas, and lower body temperature Male/Female: Cardiac arrhythmias and prolonged QT interval</td>
</tr>
<tr>
<td><strong>Epileptiform Discharges</strong></td>
<td>Male: Not investigated Female: Rhythmic cortical spike and wave discharges accompanied by behavioural arrest</td>
</tr>
</tbody>
</table>
1.3. Project Basis

With the predominant amount of research on behavioural characterization in RTT mouse models being done on the male mice there is a large gap in our knowledge of the specific deficits observed in the more clinically relevant female mice. Specifically, there has been very little research undertaken to examine daily rhythmic behaviours and thermoregulatory patterns in MeCP2-deficient female mice despite there being clinically observed deficits in RTT patients. Additionally, although epileptic seizures are a severe co-morbidity in girls with Rett syndrome, they remain largely uninvestigated in any RTT mouse model. Furthermore, the examination of relationship between MeCP2 expression and phenotypic severity in MeCP2-deficient female mice has been very superficial and not thoroughly examined. Therefore, my project aims to address these inadequacies in our knowledge of the extent of behavioural impairment in MeCP2-deficient female mice whilst hopefully furthering our understanding of the pathophysiology underlying RTT.
1.4. Specific Goals and Aims of Project

**Goal One:** Assess daily rhythmic behaviours and thermoregulatory patterns in adult female MeCP2-deficient mice.

**Specific Aims:**

- Determine if daily rhythmic patterns of core body temperature, gross motor activity, and cortical delta power are abnormal in the absence of MeCP2.
- Assess whether the relationships between motor activity, delta power, and body temperature are altered in MeCP2-deficient mice compared to wild-type mice.
- Using long duration EEG recordings, characterize the properties of the observed epileptiform discharges and study their daily patterning.

**Goal Two:** Examine the correlations between regional MeCP2 expression and specific behavioural impairments in adult female MeCP2-deficient mice.

**Specific Aims:**

- Determine MeCP2 protein expression levels in the cortex, hippocampus, cerebellum, and spinal cord of MeCP2-deficient mice.
- Assess whether MeCP2 protein levels correlate between the different brain regions in each respective mouse.
- Correlate brain region-specific MeCP2 levels against the mouse’s overall phenotypic severity, motor activity, anxiety-like behaviour, daily rhythmic behaviour, thermoregulatory deficits, and epileptiform discharge incidence rates.

**Hypothesis:** MeCP2/−/+ female mice will display altered daily rhythmic behavioural patterns and epileptiform discharges which will correlate in severity to the amount of region-specific functional MeCP2 expressed in the mouse.
Chapter 2

Materials and Methods

2.1. Animal Subjects

2.1.1. Statement of Ethics

All animal experimentation was conducted in accordance with the guidelines of the Canadian Council of Animal Care, and thoroughly reviewed and approved before implementation by the Toronto General and Western animal care committee (Protocol 1321.7). All surgery was performed under general anesthesia, and every effort was made to minimize suffering.

2.1.2. RTT Mouse Models Used

Two strains of female MeCP2-deficient mice were used in this study, the \( \text{MeCP2}^{\text{tm1.1Bird}} \) (Guy et al., 2001) and the \( \text{MeCP2}^{\text{tm2Bird}} \) (Guy et al., 2007) mouse models of RTT (both obtained from Jackson Laboratories, Bar Harbor, ME). Although different in molecular design, the \( \text{MeCP2} \) gene is disrupted in each of these lines and each displays common phenotypic progression. Therefore, they will be collectively referred to as MeCP2-deficient \( \text{MeCP2}^{\text{+/+}} \) mice. The \( \text{MeCP2}^{\text{tm1.1Bird}} \) (n=7), \( \text{MeCP2}^{\text{tm2Bird}} \) (n=4), and wild-type mice were all female, aged between 300 and 400 days, and maintained on a pure C57Bl/6 background. All animals were housed in a vivarium that was maintained at 22-23°C with a standard 12-hour light on/off cycle commencing at 6:00. For this study, Zeitgeber time (ZT) of 0 refers to the 6:00 lights-on daily time.
2.1.3. Genotyping

To determine the genotype of the mice, DNA was first isolated from collected ear punches. To do this, each freshly collected ear punch was submerged in 75 µl of 25 mM Sodium Hydroxide (NaOH) / 0.2 mM Ethylenediaminetetraacetic acid (EDTA) solution and placed in an MJ Research Thermocycler at 98 °C for 1 hour. Following this, 75 µl of 40 mM Tris(hydroxymethyl)aminomethane (Tris) Hydrochloric acid (HCL) with a pH of 5.5 was added to each sample to neutralize the solution (Truett et al., 2000). Samples were then kept at -20 °C until used as templates for Polymerase Chain Reaction (PCR) to determine mouse genotype. PCR was conducted using the MJ Research Thermocycler with a program of 35 cycles of denaturing at 94 °C for 30 seconds, annealing at 66 °C for 1 minute, and extending at 72 °C for 1 minute. For the Mecp2\textsuperscript{tm1.1Bird} mice, amplification was done using the common primer 5’-AAA TTG GGT TAC ACC GCT GA-3’, the wild-type Mecp2 allele specific primer 5’-CTG TAT CCT TGG GTC AAG CTG-3’, and the mutant Mecp2 allele specific primer 5’-CCA CCT AGC CTG CCT GTA CT-3’. With the Mecp2\textsuperscript{tm1.1Bird} primers, a single band of 465 base pairs (bp) indicated a wild-type subject while the presence of two bands, one at 465 bp the other at 240 bp, indicated a heterozygous female subject. The Mecp2\textsuperscript{tm2Bird} mice were genotyped using the common primer 5’-AAC AGT GCC AGC TGC TCT TC-3’, the wild-type Mecp2 allele specific primer 5’-CTG TAT CCT TGG GTC AAG CTG-3’, and the mutant Mecp2 allele specific primer 5’-CCA CCT AGC CTG CCT GTA CT-3’. With the Mecp2\textsuperscript{tm2Bird} primers, a single band of 379 bp indicated a wild-type subject while the presence of two bands, one at 379 bp and the other at 222 bp, indicated a heterozygous female subject. Following completion of the amplification process, 1 µl of 10x loading buffer dye was
added to the samples. The samples were then run and products were visualized on an Ethidium Bromide-stained 2% agarose gel.

2.1.4. Telemetry Implantation Surgery

Experimental mice were implanted with a mouse-specific wireless telemetry probe (TA11ETA-F10; Data Sciences International (DSI), St. Paul, MN) for recording of body temperature, general activity and EEG. The surgical implantation procedure was as previously described by El-Hayek et al. (2011) with minor modifications. Briefly, mice were anesthetized with 2% isoflurane and the wireless transmitter placed into their peritoneal cavity. Silicone elastomer insulated sensing and reference wires connecting the transmitter were orientated rostrally toward the head via a subcutaneous route. The sensing wire was soldered to an intracranial EEG polyimide-insulated stainless steel electrode with an outside diameter of 125 µm, and placed in the parietal cortex region (bregma -0.6 mm, lateral 1.5 mm, and depth 1.5 mm) with the reference wire placed at bregma -5 mm, lateral 1 mm, and depth 1.5 mm. Consistent with previous studies (Weiergräber et al., 2005), the implantation surgery caused no apparent abnormalities in the mice, and average body weights of both Mecp2+/− and wild-type mice returned to pre-operative values within 2 weeks post-surgery (32.3 g versus 32.9 g and 26.8 g versus 27.0 g for Mecp2+/− (n=11) and wild-type (n=8) respectively).

1 Chiping Wu performed all implantation surgeries on mice in this study.
2.2. Electrophysiological Data Collection and Analysis

2.2.1. Electrophysiology Data Collection

Using the implanted wireless telemetry EEG electrodes, body temperature, activity, and EEG waveforms were collected from the implanted mice for continuous 24-hour periods. Waveform data was transmitted from the TA11ETA-F10 telemetry probes to a wireless receiver (RPC-1, DSI), which passes the data through a data exchange matrix serving as a multiplexer (DSI), and was analyzed using DataQuest A.R.T. (DSI). Body temperature was acquired using the TA11ETA-F10’s thermosensor from the peritoneal cavity. Gross locomotor activity was determined by assessing the standard deviation of the wireless signal strength of the transmitter in relationship to two receiving antennae arranged perpendicularly in the RPC-1 wireless receiver. This method and arrangement has been used previously to track and measure locomotive activity in mice (Sheward et al., 2010; Yang et al., 2009). The accuracy of the system to detect ambulatory movement was further validated by visually comparing the activity output of the system with movement revealed by synchronized video recordings. Analysis of random 10 minute segments from these video data revealed that the collection program detected all of the ambulatory movements in the mice and conversely that >95% of the activity identified by the program was accompanied by visible gross movement by the mouse (n=5 mice). Both temperature and motor activity data were transmitted at a rate of 50 Hz, using a sampling frequency (analog to digital) of 250 Hz. The EEG waveform was transmitted at 200 Hz and sampled at 1kHz.
2.2.2. Characterization of Cortical Epileptiform Discharge Events

The collected 24 hour EEG traces were visually inspected to confirm and quantify the presence of discharge activity as described previously (D’Cruz et al., 2010). In brief, a discharge event was defined as having amplitudes of at least 1.5-fold background, durations of at least 0.4 seconds, and a frequency of between 6 and 10 Hz. Two genotype-blinded investigators independently assessed EEG activity, and the individual counts were averaged. The overall concordance between these individuals was 86.4%, and these differences were averaged for final analysis of discharge incidence rate and the times of discharge occurrence over the 24-hour cycle. Having confirmed the presence of discharge activity using established manual criteria (D’Cruz et al., 2010), we then developed an automated method to characterize the duration and frequency components of the discharges. For this, a 6-10 Hz Finite Impulse Response (FIR) band pass filter was applied to specifically isolate the frequency band associated with the discharges. The envelope of the filtered signal was produced by convolution of the square of the filtered data with a Gaussian kernel of 200-point aperture (Suffczynski et al., 2010). This envelope peaks whenever strong 6-10 Hz activity is present. As normal cortical EEG signals rarely display high-amplitude rhythmic spiking within this frequency, the envelope peak reflects discharge events (Figure 3). To determine discharge durations, the left and right inflection points of detected events were used to find the start and end points respectively. The inflection points were computed by convolving the envelope with the derivative of the Gaussian kernel as above.

The DataQuest A.R.T. program (DSI) was used to generate total spectral plots over the 24-hour period for individual mice. This was achieved by using the periodogram
analysis function in the DataQuest A.R.T. program which estimates the spectral density of a signal using a Fast Fourier Transformation (FFT) with a window overlap of 50%. Additional time-frequency analyses were also conducted on 1 minute EEG segments collected during active and inactive states of the mice using the continuous wavelet transform (CWT) found in the Matlab digital signal processing toolbox. The basis function used in the CWT analysis was the Morlet mother wavelet (Chiu et al., 2006; Grossmann and Morlet, 1984), which is commonly used in EEG analyses. To minimize the issue of scaling, the analysis was divided into low frequencies (0.5 - 30Hz) and high frequencies (30 - 80Hz), with 0.5 Hz step size.

2.2.3. Recognition of Periodic Variations in EEG, Motor Activity, and Body Temperature

EEG signals within the delta band (0.5-4 Hz) (Buzsáki, 2006) were extracted by applying a series of steps. First, the data were pre-processed by removing segments indicative of movement artifacts (characterized by voltages higher than 0.5V), and the 0.25 second time period preceding and succeeding these events. Then, a FIR band pass filter with an order of 1000 was applied to isolate specifically the delta band. Delta power was obtained by squaring the delta band signal, and then averaging these values over 30 second intervals so the resulting value aligns with the movement activity and temperature signals (which were also recorded at 30 second sampling periods) by the data acquisition system. The Pearson’s product-moment correlations between delta power, motor activity, and core body temperature were conducted using smoothed versions of these raw 30 second interval data. The smoothing function employed was the 50-point FFT in OriginPro 6.1 (OriginLab Corporation, Northampton, MA). To then discern the daily
patterning of these three signals, each was normalized to have 0 mean and variance of 1, and a Gaussian-based kernel with aperture 50 was applied on all three signals generating an envelope of the signals. A threshold of 0 was then applied to discretize the signals into two different states (Figure 4). The delta power parameter was discretized into delta and non-delta states with a ‘complete delta cycle’ being defined as a state of delta followed by state of non-delta, where each individual state has a duration of at least 15 minutes. Similarly, ‘mobility cycles’ and ‘body temperature cycles’ were defined as the combination of a consecutive active and an inactive state, or consecutive high body temperature and a low body temperature states, respectively.

\footnote{Sinisa Colic developed the automated detection systems for both daily behavioural rhythm patternings and epileptiform discharges. He was also responsible for all data generated through the use of this developed method.}
Figure 3

A

B

C

rate of change

0 1 2 3 4 5 6 7 8 9 10

40
Figure 3. Automated detection of epileptiform discharges.

Panel A: Raw 10-second EEG waveform segment collected from a representative $MeCP2^{+/+}$ mouse displaying 2 epileptiform discharges as determined and confirmed by visual inspection (Red lines represent the start and end of the respective discharge event). Panel B: Resulting envelope of the EEG waveform in Panel A after band pass filtering the signal through a 6-10 Hz FIR filter and then convoluting the square of this filtered data with a Gaussian kernel of 200 point aperture (Red lines represent the start and end of the respective discharge events, the green line represents the envelope of the black 6-10 Hz FIR band pass filtered signal). Panel C: Resulting derivative of the convolved envelope signal presented in Panel B used to determine the start and end of the discharge event. The red lines denote the left and right inflection points used to determine the start and end of the discharges, respectively.
Figure 4

A

\begin{itemize}
  \item[i)]\hspace{1cm} x10^{-4}
  \item[ii)]\hspace{1cm} x10^{-4}
\end{itemize}

\begin{figure}[h]
\centering
\begin{tabular}{c}
\begin{tabular}{c}
\textbf{Wild-type}
\end{tabular}
\end{tabular}
\end{figure}

B

\begin{itemize}
  \item[i)]\hspace{1cm} x10^{-5}
  \item[ii)]\hspace{1cm} x10^{-5}
\end{itemize}

\begin{figure}[h]
\centering
\begin{tabular}{c}
\begin{tabular}{c}
\textbf{MeCP2^{-/-}}
\end{tabular}
\end{tabular}
\end{figure}
Figure 4 Cont’d

C i) Wild-type

Index of mobility (arb. units)

ZT (hrs)

C ii)

Index of mobility (arb. units)

ZT (hrs)

D i) MeCP2−/−

Index of mobility (arb. units)

ZT (hrs)

D ii)

Index of mobility (arb. units)

ZT (hrs)
Figure 4 Cont’d

E

i)

Temperature (°C)

0 2 4 6 8 10 12
ZT (hrs)

Wild-type

ii)

Temperature (°C)

12 14 16 18 20 22 0
ZT (hrs)

F

i)

Temperature (°C)

0 2 4 6 8 10 12
ZT (hrs)

MeCP2^+/+

ii)

Temperature (°C)

12 14 16 18 20 22 0
ZT (hrs)
**Figure 4. Recognition of periodic variations in EEG, gross motor activity, and core body temperature.**

Panels A and B: Representative traces of cortical delta power patterning over the light (i) and dark (ii) phases of a 24 hour day in a wild-type (A) and a *Mecp2<sup>+/−</sup>* (B) mouse. Shaded regions denote areas classified as high delta states and non-shaded regions denote areas classified as low (non) delta states. Panels C and D: Representative traces of mobility patterning over a 24 hour day in a wild-type (C) and a *Mecp2<sup>+/−</sup>* (D) mouse. Shaded regions denote areas classified as mobile behavioural states whereas non-shaded regions denote areas classified as inactive behavioural states. Panels E and F: Representative traces of core body temperature patterning over a 24 hour day in a wild-type (E) and a *Mecp2<sup>+/−</sup>* (F) mouse. Shaded regions denote areas where body temperature was above the daily mean value, whereas non-shaded regions denote areas where body temperature was below the mean.
2.3. Behavioural Assessments

2.3.1. Open Field Apparatus

*Mecp2*+/− mouse performance in the open field behaviour test was conducted between the hours of 8:00 and 14:00 to minimize the effects of circadian rhythms. All behavioural tests were performed in a sound-attenuating room in the presence of 65 decibel (dB) ambient white noise to recapitulate the environment in the mouse housing room and *Mecp2*+/− animal behaviour was assessed 2 months prior to sacrifice of the animals. An automated movement detection system (AM1053 Activity Monitoring System; Linton Instrumentation, UK) was used to assess open field movement behaviour. Mice were placed individually into an apparatus consisting of a plexiglass box (20 x 30 cm²) surrounded by a housing frame (45 x 25 cm²) that houses infrared beams forming a grid across two levels to detect motion (Jugloff et al., 2008; Visanji et al., 2006). When the mouse moves in the open field apparatus, various infrared beams are broken depending on the type of movement performed by the mouse. Based on the location, level, and speed in which the beams are broken in the apparatus, different types of behaviour can be examined. Total beam breaks are indicative of overall locomotive capabilities of the mouse, whereas the total amount of rearing displayed by the mouse is representative of exploratory behaviour (occurs when the mouse raises its forepaws from the floor breaking the second tier of infrared beams). Additionally, anxiety-like behaviour can be assessed using the open field apparatus by monitoring the total amount of rearing performed by the mouse in the centre of the arena. Activity in the open field arena was recorded during 1 hour test sessions and odors were controlled for by wiping the interior of the box with 70 % ethanol solution after each test.
2.3.2. Light and Dark Placement Preference Test

Anxiety-like behaviour of Mecp2+/− female mice was examined using a modified light and dark placement preference behavioural test, based on the procedure previously described by Bouwknecht and Paylor (2002). On the day of testing, mice are first transferred from the colony room to the sound-attenuated behavioural testing room where they are left to acclimatize to their surroundings in the presence of white noise. Mice were then placed in a plexiglass apparatus containing a 20 cm x 14 cm dark chamber (0.5 lux) with a 4 cm² opening to a bright 20 cm x 28 cm open area illuminated by a fluorescent lamp (1000 lux). Mice were initially placed in the light area of apparatus facing the opening to the dark chamber and mouse movement was recorded for 5 minutes. The number and duration of risk assessments were recorded for each mouse over the 5 minutes, where fewer risk assessments are indicative of increased anxiety-like behaviour. Risk assessments were classified as when a mouse would poke its head into the light compartment while keeping its paws in the dark compartment and risk assessment duration was classified as the cumulative time the mouse spent poking its head into the light compartment over the 5 minutes.

2.3.3. Overall Phenotypic Severity Scores

Overall phenotypic severity of Mecp2+/− mice was assessed using a 12-point symptom severity score as previously described (Guy et al., 2007). This scale assigns either 0 (symptom was not present), 1 (symptom is present), or 2 (symptom is severe) in 6 categories: inertia, gait, tremor, breathing, hind limb clasp, and general well-being. To score the mice, each mouse was brought out of its cage and examined under a bio-safety
cabinet. First, mice were suspended by their tail to observe the extent of hind limb clasping; both legs outstretched indicated a score of 0, momentary clasping of one or both legs indicated a score of 1, and prolonged clasping of both legs was scored as 2. Mice were then placed on the floor of the bio-safety cabinet and the extent of movement performed by the mouse was examined. If the mouse displayed a lot of exploratory movement it was given a score of 0, if it displayed moderate amounts of movement a score of 1 was given, and if the mouse did not move at all it was given a score of 2. While moving about the mouse’s gait was also examined, if a mouse displayed double steps, splayed out steps, and seemed to be sliding its feet it was awarded a score of 2, if there were only double steps observed a score of 1 was given, absence of these impairments was awarded a score of 0. Additionally, in the case where the mouse performed no movement in which to assess gait even after sufficient motivation and prompting, a score of 2 was awarded as it was evident the mouse was unable to move due to severe gait impairment. Breathing was scored by visual examination of the breathing pattern of the mouse, if apneas and irregular breathing patterns were observed the mouse was awarded a score of 2, if the mouse just displayed irregular breathing it was given a 1, rhythmic breathing was awarded a 0. The presence of resting tremors was visually assessed and if the mouse displayed constant tremors it was given a 2, intermittent tremors were awarded a score of 1, and absence was given a 0. Finally, general well-being was assessed by examining the overall condition of the mouse. If the mouse had prominent disheveled fur and periocular lesions it was awarded a score of 2, mice with moderate amounts of disheveled fur were given a score of 1, and if the mouse was well groomed was awarded a score of 0.
2.4. Western Blots

2.4.1. Tissue Harvesting and Preparation

Wild-type and MeCP2+/− mice were sacrificed by isoflurane overdose and their brain and spinal cord isolated rapidly on ice. Cerebral cortices, cerebellum, hippocampi, and the spinal cord were then dissected, and frozen on dry ice. These tissues were then partially thawed, and homogenized by sequential passage through 20, 23, and 25½ gauge syringe needles in 300 µl of Radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCL[pH 7.5], 150 mM Sodium Chloride (NaCl), 1% Nonyl Phenoxypolyethoxylethanol (NP40), 2 mM EDTA, 0.5% sodium deoxycholate, and 0.1% Sodium Dodecyl Sulfate (SDS)) supplemented with a mixture of protease inhibitors (Phenylmethanesulfonylfluoride (PMSF) 40 ng/ml, Antipain 2 ng/ml, PepstatinA 2 ng/ml, Leupeptin 20 ng/ml, Aprotinin 20 ng/ml, and MDL28170 20 ng/ml), and the crude homogenate centrifuged at 12,000 x g for 5 minutes to remove insoluble material. Aliquots of the remaining supernatant were then collected, and the samples stored at -80°C until use.

2.4.2. Protein Concentration Measurements

Protein concentrations of individual samples were determined using the Folin method protein assay (Bio-Rad, Hercules, CA, Cat # 500-0116) and absorbance values were measured at 630 nm wavelength using a spectrophotometer (BioTek EL311 AutoReader). The recorded values were normalized to a blank which consisted of only RIPA buffer and Folin reagents. Six standard protein concentrations were used to generate a standard curve, in which the absorbance values of the samples were compared...
against to determine overall protein concentration. Three replicates were used for each sample and then averaged to determine the final protein concentration of each sample.

2.4.3. Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Samples were first prepared for gel electrophoresis by adding loading buffer (50 mM Tris-HCl[pH 6.8], 2% SDS, 10% Glycerol, 1% Beta-mercaptoethanol, 12.5 mM EDTA, and 0.02% Bromophenol Blue) and denaturing the proteins by heating the samples at 95 °C for 5 minutes. Following this, 20 µg of protein from each sample was loaded and resolved on a 5% stacking / 10% resolving acrylamide gel in Tris-glycine Laemelli running buffer (25 mM Tris, 192 mM Glycine, and 0.1% SDS) at a constant voltage of 130 V for 2 hours as previously described (Asaka et al., 2006). The resolved proteins were then transferred electrophoretically to a nitrocellulose membrane in standard transfer buffer (25 mM Tris, 192 mM Glycine, 20% Methanol) overnight at 4 °C using a constant voltage of 35 V. After the transfer, the membrane was pre-hybridized for 2 hours at room temperature with blocking solution, 5% non-fat dry milk solution dissolved in TBST washing buffer (10 mM Tris, 150 mM NaCl, 0.05% Polyoxyethylene (20) sorbitan monolaurate (Tween-20)). The blots were then incubated overnight at 4 °C with primary antibody in blocking solution. The primary antibodies used were anti-MeCP2 at a dilution of 1:1,000 to blocking solution (Cell Signaling Technology, Danvers, MA, Cat # 3456S) and anti-Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) at a dilution of 1:15,000 to blocking solution (Chemicon, Billerica, MA, Cat # MAB374). Following primary antibody incubation, the blots were washed in TBST (3 x 20 minutes washes) and then incubated for 2 hours with horseradish peroxidase (HRP)-
linked secondary antibodies at room temperature. The secondary antibodies used were anti-rabbit (against anti-MeCP2) and anti-mouse (against anti-GAPDH), both at a dilution of 1:5,000 to blocking solution (GE Healthcare, Buckinghamshire, England, Cat # NA934 [anti-rabbit], Cat # NA931 [anti-mouse]). Following further extensive washing in TBST, specific immunoreactivity was visualized by enhanced chemiluminescence (GE Healthcare, Amersham ECL Western Blotting Detection Reagents, Cat # RPN2106).

2.4.4. Immunoblot Quantification and Analysis

Immunoreactive protein levels were determined using densitometric analysis. Blots were scanned and visualized using Fluor-S MultiImager (Bio-Rad, Hercules, CA) and the density of the bands was determined using the rectangular density function in the Quantity One (Bio-Rad) analysis program. Film background was subtracted from the calculated specific immunoreactive product to obtain the overall protein density values. MeCP2 protein levels were then normalized to their corresponding GAPDH protein levels to normalize for protein loading discrepancies. To allow direct comparisons of MeCP2 expression levels in different brain regions across our cohort of MeCP2-deficient mice, we selected mouse 7 to serve as a common reference. The GAPDH-normalized MeCP2 protein values for each brain region were then normalized to the GAPDH-normalized MeCP2 level in the same brain region of Mouse 7. As such, the normalized MeCP2 levels in Mouse 7 are recorded as 100% in each region.
2.5. Statistical Analysis

Student’s t-tests were used for direct comparisons between two groups. For comparisons between multiple groups, one-way ANOVA with Bonferroni post hoc correction for multiple comparisons was utilized. F-tests were used to compare the equality of two variances between groups. For comparing the correlative strength between two groups, Pearson’s product moment correlation coefficient, a determiner of linear dependency between two variables, was employed. Significance was set at p<0.05. Mean and standard error of the mean (SEM) are presented throughout the text and figures.
Chapter 3

Results

3.1. Daily Rhythmic Behaviours and Thermoregulatory Patterns are Disrupted in Adult Female MeCP2-Deficient Mice

3.1.1. The General Properties of Cortical EEG Activity are Preserved in Mecp2\(^{+/+}\) mice

Consistent with previous observations by our laboratory (D’Cruz et al., 2010), the general neocortical EEG signals in adult Mecp2\(^{+/+}\) mice did not display overt differences from adult female wild-type mice. Waveforms with elevated amplitude and slow frequency (0.5-4 Hz, delta band) were evident during immobile and sleep-like behaviour, while lower amplitude higher frequency activity was seen during periods of movement or exploration. CWT time-frequency analyses (Chiu et al., 2006) of these cortical EEG activities revealed no qualitative differences in the frequency powers of wild-type and Mecp2\(^{+/+}\) mice within the 0.5-80Hz spectrum during either the active or inactive states of behaviour (Figure 5A-D). Additionally, time-frequency analysis of Mecp2\(^{+/+}\) and wild-type 24-hour EEG waveforms using a Fast Fourier Transformation revealed the overall power spectrum distributions between the two groups was preserved (Figure 5E, F), and analysis of specific waveforms such as the delta band (0.5-4 Hz), alpha band (8-12 Hz) and beta band (15-30 Hz), across the full 24-hour day also revealed no significant differences in overall power between the groups (Figure 5G).

Figure 5

A  i) Wild-type: Active

ii) iii)

B  i) MeCP2\textsuperscript{−}: Active

ii) iii)
Figure 5 Cont’d

C  i) Wild-type: Inactive

![Graph](image)

ii) ![Graph](image)

iii) ![Graph](image)

D  i) MeCP2\(^+\): Inactive

![Graph](image)

ii) ![Graph](image)

iii) ![Graph](image)
Figure 5 Cont’d

E

F

G

Normalized Power (% mV²/Hz)

Frequency (Hz)

Normalized Power (% mV²/Hz)

Frequency (Hz)

Normalized Power (% mV²/Hz)

Frequency Range

0.5-4 Hz  8-12 Hz  15-30 Hz
Figure 5. The general EEG waveform properties of the *Mecp2* mouse cortex are similar to wild-type.

Panels A-D: Representative examples of a 1 minute segment of raw EEG activity (i) taken from wild-type (A and C) and *Mecp2* mice (B and D) during mobility (A and B) and during inactivity (C and D). Shown below each raw EEG trace is the corresponding wavelet transformation showing the spectrum of frequency power for the 0.5-30 Hz range (ii) and for the 30-80 Hz range (iii). Panels E and F: Average normalized time-frequency power spectrum plots of wild-type (E) and *Mecp2* (F) mice. Panel G: Average power of the delta band (0.5-4 Hz), the alpha band (8-12 Hz) and beta band (15-30 Hz) in wild-type and *Mecp2* mice normalized to peak power for each respective mouse. Histograms are plotted as mean ± SEM. Asterisks denote statistical significance *p*<0.05, student’s unpaired t-test for n=11 *Mecp2* mice and n=8 wild-type mice.
3.1.2. Mecp2+/+ Mice Display Alterations in Their Daily Pattern of Cortical Delta Wave Activity

The presence of delta slow wave cortical EEG waveforms is often used as an indicator of sleep in wild-type rodents (Franken et al., 1998). Analysis of smoothed cortical delta power (as derived from the EEG waveforms, Figure 6) in wild-type mice revealed clearly defined patterns of rhythmicity over the 24-hour period (Figure 7A). In contrast, the daily patterns of delta power in Mecp2+/+ mice were more erratic in periodicity and duration (Figure 7B). Comparison of wild-type, and Mecp2+/+ mice revealed a significant decrease in the average number of delta cycles over a 24-hour period (12 hours light, 12 hours dark, Figure 7C). Mecp2+/+ mice displayed an average of 7.6±0.6 delta cycles compared to 11.3±0.7 in controls (p=0.001). This overall decrease was equivalently diminished in both phases of the 24-hour day (p<0.01, for each respectively, Figure 7C). Further, the average duration of the non-delta state of each cycle period was significantly longer in Mecp2+/+ mice than wild-type mice (1.5±0.3 hours versus 0.75±0.1 hours, p<0.05, Figure 7D). This increase in non-delta state duration was present during both the light and dark phases of the 24-hour day, and consistent with the preferential nocturnal behaviour of mice, both Mecp2+/+ and wild-type mice displayed greater non-delta time durations in the dark phase of the 24-hour day.
Figure 6

A  Wild-type: Light

B  Wild-type: Dark

C  MeCP2^{+/-}: Light

D  MeCP2^{+/-}: Dark
Figure 6. Illustration of smoothed data generated from raw delta power traces.

Panels A-D: Representative traces of raw cortical delta power (grey line) and the resulting smoothed data (black line) as generated using the 50-point Fast Fourier Transformation (FFT) smoothing function in OriginPro 6.1 (OriginLab Corporation, Northampton, MA) for a wild-type (A and B) and a MeCP2+/− (C and D) mouse during the day (A and C) and night (B and D) phases of the 24-hour day.
Figure 7

A

Wild-type

Delta Power (mV²/Hz)

0
5.0x10⁻⁵
1.0x10⁻⁴
1.5x10⁻⁴
2.0x10⁻⁴

ZT (hrs)

B

MeCP2⁻/⁺

Delta Power (mV²/Hz)

0
5.0x10⁻⁵
1.0x10⁻⁴
1.5x10⁻⁴

ZT (hrs)

C

Wild-type

MeCP2⁻/⁺

Number of Cycles

0
5
10
15

Total Light Dark

D

Average State Duration (h)

0
0.5
1.0
1.5
2.0

Total Light Dark

*
Figure 7. *Mecp2*+ mice display altered daily delta cycle periodicity and duration.

Panels A and B: Representative traces of delta power patterning over 24 hours in a wild-type (A) and a *Mecp2*+ (B) mouse. The dark phase (ZT 12-24) is shaded in these plots. Panels C and D: Histograms showing the average number of delta cycles (C), and the average duration of time spent in a non-delta state per delta cycle (D). For panels C and D, the total 24-hour data set is shown with the data for either the light (ZT 0-12) or dark (ZT 12-24) phases specifically. Shown are the mean ± SEM for n=11 *Mecp2*+ and n=8 wild-type mice. Asterisks denote statistical significance at *p*<0.05, one-way ANOVA with Bonferroni post hoc correction for multiple comparisons.
3.1.3. *Mecp2*/*+ Mice Display Alterations in Daily Cyclic Mobility Patterns

In contrast to the alterations in delta power periodic patterning, examination of smoothed mobility patterns (Figure 8) failed to reveal differences in cycle number between wild-type (Figure 9A) and *Mecp2*/*+* mice (Figure 9B) over the 24-hour day. *Mecp2*/*+* mice displayed an average of 9.0±0.8 total mobility cycles over the 24-hour day, while wild-type mice displayed an average of 9.0±0.7 cycles (Figure 9C). However, although the number of mobility cycles was preserved, the distribution of time in the active phase versus the inactive phase of these cycles differed between *Mecp2*/*+* and wild-type mice. Specifically, the average duration of the active state of a cycle was significantly decreased in *Mecp2*/*+* mice over the 24-hour day (0.52±0.03 hours versus 0.66±0.04 hours, \( p<0.05 \), Figure 9D), with the difference being predominant in the dark phase of the 24-hour cycle. Wild-type mice showed longer active state durations in the dark relative to the light phases (0.78±0.06 hours versus 0.54±0.02 hours in dark and light respectively, \( p<0.005 \)), while *Mecp2*/*+* mice exhibited similar active state durations in both light and dark phases (0.52±0.04 hours versus 0.50±0.05 hours in dark and light respectively, Figure 9D).
Figure 8

A

Wild-type: Light

B

Wild-type: Dark

C

MeCP2\textsuperscript{+/-}: Light

D

MeCP2\textsuperscript{+/-}: Dark
Figure 8. Illustration of smoothed data generated raw mobility traces.

Panels A-D: Representative traces of raw mobility (grey line) and the resulting smoothed data (black line) as generated using the 50-point FFT smoothing function in OriginPro 6.1 (OriginLab Corporation) for a wild-type (A and B) and a MeCP2+/− (C and D) mouse during the day (A and C) and night (B and D) phases of the 24-hour day.
Figure 9

A

Wild-type

B

MeCP2\(^{+/+}\)

C

D

Wild-type

MeCP2\(^{+/+}\)

Number of Cycles

Average State Duration (h)

Total

Light

Dark

Total

Light

Dark
Figure 9. Mecp2\textsuperscript{+/+} mice display abnormal cycles of daily activity.

Panels A and B: Representative traces of activity patterning over 24 hours in a wild-type (A) and a Mecp2\textsuperscript{+/+} (B) mouse. The dark phase is shaded. Panels C and D: Histograms showing the average number of mobility cycles (C), and the average duration of time spent in an active state per mobility cycle (D). For panels C and D, the total 24-hour data set is shown with the results specific for either the light or dark phases. Shown are the mean ± SEM for n=11 Mecp2\textsuperscript{+/+} and n=8 wild-type mice. Asterisks denote statistical significance at \( p<0.05 \), one-way ANOVA with Bonferroni post hoc correction for multiple comparisons.
3.1.4. Mecp2\(^{+/+}\) Mice Possess Altered Home Cage Mobility Profiles

The reduced active state duration in Mecp2\(^{+/+}\) mice suggests that they spend more time in the awake-immobile state than the awake-active state relative to wild-type mice. Analysis of the raw movement profiles revealed a significant reduction in the total amount of mobility (as deduced by changes in strength of the telemetry signal at the receiver) between Mecp2\(^{+/+}\) mice and wild-type mice (121±7 versus 204±20 mobility counts, respectively \(p<0.005\)). Further, the overall time spent by Mecp2\(^{+/+}\) mice moving in their home cages over a 24-hour period was significantly reduced compared to age-matched wild-type mice (694±31 versus 931±43 segments containing mobility, \(p<0.001\), Figure 10A, B). In addition to total mobility differences, the average rate of movement by the Mecp2\(^{+/+}\) mice was also diminished relative to wild-type (0.17±0.01 versus 0.22±0.01 mobility counts/sec, \(p<0.05\)), and this effect was the most pronounced in the dark phase of the day (Figure 10C).
Figure 10

A

B

C

[Bar charts showing mobility data for Total, Light, and Dark conditions with error bars and asterisks indicating statistical significance.]
Figure 10. *Mecp2*<sup>+/−</sup> mice display mobility deficits in their home cage environment.

Panels A-C: Histograms showing the home-cage activity parameters of *Mecp2*<sup>+/−</sup> and wild-type mice over the full 24 hours, and for the light and dark phases of the day specifically. Panel A shows the total amount of mobility of mice over 24 hours. Panel B shows the number of 30-second segments throughout the day in which *Mecp2*<sup>+/−</sup> and wild-type mice displayed mobility. Panel C shows the average rate of movement (magnitude of movement per second) performed by *Mecp2*<sup>+/−</sup> and wild-type mice. Shown are the mean ± SEM for n=11 *Mecp2*<sup>+/−</sup> and n=8 wild-type mice. Asterisks denote statistical significance at $p<0.05$, one-way ANOVA with Bonferroni *post hoc* correction for multiple comparisons.
3.1.5. The Inverse Correlation of Delta Power and Behavioural Activity is Disrupted in Mecp2\textsuperscript{+/+} Mice

In wild-type mice, there was a strong inverse correlation between mobility and cortical delta power (Figure 11A). This was not the case in Mecp2\textsuperscript{+/+} mice (Figure 11B). The strength of the Pearson’s product-moment correlation coefficient (a measure of linear dependency) revealed a strong inverse correlation (average $r = -0.75$) between delta power and movement in wild-type mice, consistent with delta power serving as a good predictor for sleep / immobility in wild-type mice (Franken et al., 1998). However, the Pearson’s correlation coefficient for delta power and movement in Mecp2\textsuperscript{+/+} mice was significantly weaker (average $r = -0.42$), indicating that delta power is not a good predictor for immobile or sleep states in Mecp2\textsuperscript{+/+} mice (Figure 11C). In fact, as shown in Figure 11B, instances of high delta power concomitant with mobility were frequently observed in Mecp2\textsuperscript{+/+} mice.
Figure 11

A

Wild-type

Index of mobility (arb. units)

0.00 0.05 0.10 0.15

Time (hr)

0 2 4 6

Delta Power (mV^2/Hz)

---

Motor Counts

B

MeCP2^-/-

Index of mobility (arb. units)

0.00 0.05 0.10 0.15

Time (hr)

0 2 4 6

Delta Power (mV^2/Hz)

---

C

Pearson Coefficient (r)

-1.0 -0.5 0.0 0.5 1.0

Wild-type Total

MeCP2^-/- Total

Wild-type Light

MeCP2^-/- Light

Wild-type Dark

MeCP2^-/- Dark

*
Figure 11. The normal correlation between cortical delta power and mobility is altered in Mecp2<sup>+/−</sup> mice.

Panels A and B: Representative traces of activity and delta power parameters over a 6 hour period in the light phase of the day for a wild-type (A) and a Mecp2<sup>+/−</sup> (B) mouse. For each, the solid black line denotes mobility while the grey dotted line denotes delta power. Panel C: Scatter plots showing the Pearson’s product-moment correlation coefficients for delta power compared to activity in Mecp2<sup>+/−</sup> and wild-type mice. Each point represents the daily correlative strength for a single subject. The bar on the scatter plot indicates the mean for each set. Asterisks denote statistical significance (p<0.05) between the indicated groups (student’s unpaired t-test). n=11 Mecp2<sup>+/−</sup> and n=7 wild-type mice, respectively.
3.1.6. Mecp2<sup>+/+</sup> Mice Display Impaired Body Temperature Patterning and Regulation

The patterns of cyclic body temperature fluctuations (derived from smoothed raw data, Figure 12) also revealed differences between wild-type (Figure 13A) and Mecp<sup>2+/+</sup> mice (Figure 13B). Mecp<sup>2+/+</sup> mice displayed fewer temperature cycles per day than wild-type mice (6.8±0.5 versus 9.8±0.7, Figure 13C), and an increase in the average duration of time spent in the high phase of their temperature cycle relative to wild-type mice (1.47±0.13 versus 0.95±0.09 hours, respectively, Figure 13D). In addition to having impaired periodic rhythmic patterns, the average daily minimal temperature, and the average daily maximal temperature of Mecp<sup>2+/+</sup> mice were each significantly lower than wild-type (33.9±0.7°C versus 35.6±0.2°C respectively, for minimum, p<0.05; and 37.9±0.2°C versus 38.5±0.1°C respectively, for maximum, p<0.05). Consistently, the core body temperature range of Mecp<sup>2+/+</sup> mice had higher variance than wild-type mice over the 24-hour day (4.06°C<sup>2</sup> range versus 0.31°C<sup>2</sup> range, respectively, p<0.005, Figure 14A). Moreover, during periods of mobility and inactivity specifically, the temperature of Mecp<sup>2+/+</sup> mice was significantly lower than that of wild-type mice (36.6±0.2°C versus 37.4±0.1°C for mobile states and 35.8±0.3°C versus 36.8±0.1°C for inactive states, p<0.005 and p<0.05 respectively, Figure 14B, C), and the correlation coefficient between movement and body temperature in the Mecp<sup>2+/+</sup> mice was substantially weaker than that of the wild-type mice (0.76 versus 0.54, respectively, p=0.001, Figure 14D). Collectively, these results indicate Mecp<sup>2+/+</sup> mice display an overall reduction in core body temperature throughout the day, and that their homeostatic regulation of body temperature is impaired.
Figure 12

A

Wild-type: Light

Temperature (°C)

B

Wild-type: Dark

Temperature (°C)

C

MeCP2⁻/⁺: Light

Temperature (°C)

D

MeCP2⁻/⁺: Dark

Temperature (°C)
Figure 12. Illustration of smoothed data generated from raw body temperature traces.

Panels A-D: Representative traces of raw core body temperature (grey line) and the resulting smoothed data (black line) as generated using the 50-point FFT smoothing function in OriginPro 6.1 (OriginLab Corporation) for a wild-type (A and B) and a $MeCP2^{+/+}$ (C and D) mouse during the day (A and C) and night (B and D) phases of the 24-hour day.
Figure 13

A

Wild-type

Temperature (°C)

30 32 34 36 38 40

ZT (hrs) 0 6 12 18 24

B

MeCP2/+'

Temperature (°C)

30 32 34 36 38 40

ZT (hrs) 0 6 12 18 24

C

[Bar graph showing number of cycles for Wild-type and MeCP2/+']

D

[Bar graph showing number of cycles for Total, Light, and Dark conditions]

* Significant difference
Figure 13. The normal pattern of body temperature cycling is altered in *Mecp2*+/− mice.

Panels A and B: Representative traces of daily body temperature over 24 hours in a wild-type (A) and a *Mecp2*+/− (B) mouse. The dark phase of the day is shaded on each panel. Panels C and D: Histograms showing the average number of body temperature cycles (C), and the average duration of time spent in the top half of the full temperature range for each mouse across the day (D). For panels C and D, the total 24-hour data set is shown along with the results specific for either the light or dark phases. Shown are the mean ± SEM for n=11 *Mecp2*+/− and n=8 wild-type mice. Asterisks denote statistical significance at *p*<0.05, one-way ANOVA with Bonferroni post hoc correction for multiple comparisons.
Figure 14

A

Temperature Range (°C)

Wild-type  MeCP2+/+

B

Active Temperature (°C)

Total  Light  Dark

Wild-type  MeCP2+/+

C

Inactive Temperature (°C)

Total  Light  Dark

Wild-type  MeCP2+/+

D

Pearson Coefficient (r)

Wild-type Total  Wild-type Light  Wild-type Dark

MeCP2+/+ Total  MeCP2+/+ Light  MeCP2+/+ Dark
Figure 14. Core body temperature regulation is altered in MeCP2+/− mice.

Panel A: Scatter plot showing the range of core body temperature in MeCP2+/− and wild-type mice. Each point represents the absolute range (min to max during the 24-hour day) of core body temperature for an individual mouse. On Panel A, # denotes p<0.05 as determined using an F-test for the equality of two variances. Panel B and C: Histograms showing the mean ± SEM of the active body temperature of MeCP2+/− and wild-type mice (B), and their average inactive body temperature (C) throughout the day, or specifically during the light or dark phases. Panel D: Scatter plot showing the Pearson’s product-moment correlation coefficients for mobility and temperature in MeCP2+/− and wild-type mice. Each point represents the daily correlative strength between mobility and temperature for a single subject. The bar on the scatter plot indicates the mean for each set. Asterisks denote statistical significance (p<0.05) between the indicated groups (student’s unpaired t-test). n=11 MeCP2+/− mice and n=7 wild-type mice.
3.1.7. *Mecp2*/+ Mice Display Spontaneous Cortical Epileptiform Discharge Activity

Raw EEG waveform data was examined from *Mecp2*/+ mice to determine the prevalence and distribution of epileptiform discharges throughout the 24-hour period. For these assessments, a discharge event was defined as a high amplitude rhythmic waveform lasting at least 0.4 seconds with a frequency between 6 and 10 Hz (Figure 15A). No discharge activity was detected in any of the wild-type mice examined (n=8). Cortical EEG discharges were observed in 8 of 11 *Mecp2*/+ mice. In these mutants, the average number of cortical epileptiform discharges per hour over a 24-hour period was 10.7±1.6 (Figure 15B). The average duration of the discharge events was 0.76±0.01 seconds, and the average frequency of the discharges was 8.6±0.02 Hz (Figure 15C, D). While spontaneous convulsions were not observed in any of the *Mecp2*/+ mice, cortical discharge activity was associated with behavioural freezing, which often lasted longer than the duration of the discharge. Analysis of discharge activity during the light and dark phases of the day failed to reveal any significant differences: the incidence rate of the discharges, their average duration, and their average frequency did not significantly differ during the light or dark phases.
Figure 15

A

i) 

![Voltage vs Time](image1)

ii) 

![Frequency vs Time](image2)

iii) 

![Frequency vs Time](image3)

B

![Bar Chart: Discharges per Hour](image4)

C

![Bar Chart: Discharge Duration](image5)

D

![Bar Chart: Discharge Frequency](image6)
Figure 15. Properties of epileptiform discharges in Mecp2+/− mice.

Panel A: Representative example of a 10 second segment of raw EEG activity (i) illustrating a typical discharge event in a Mecp2+/− mouse, and the corresponding wavelet transformation showing the spectrum of frequency power for the 0.5-30 Hz range (ii) and for the 30-80 Hz range (iii). Panels B-D: Histograms showing the mean ± SEM of the discharge rate per hour (B), the average discharge duration (C), and the average frequency component of all the discharges (D) in Mecp2+/− mice. Presented on each histogram is the total over the 24-hour period, and the data stratified for specifically light and dark phases. No statistically significant differences in discharge activity, duration, or frequency were seen between the light and day phases (student’s paired t-test, n=8 Mecp2+/− mice).
3.1.8. Cortical Epileptiform Discharge Activity Predomimates During the Active State of a Mobility Cycle

To assess whether cortical discharge activity occurred randomly throughout the day, or was preferentially seen during certain behavioural states, we compared discharge activity across active and inactive states, and in periods of high and low core body temperature. These assessments revealed that significantly higher discharge activity was found in $\text{MeCP2}^{+/+}$ mice during the active phase of their behavioural mobility cycle during the entire day (22.0±4.0 versus 5.4±1.0 discharges, $p<0.005$, Figure 16A, B), and during the light and dark phases of the day, specifically. In contrast, no significant association between core body temperature and discharge activity was seen. For this, we compared discharge activity in mice during times when their core body temperature was within the top or bottom 25% range of the full 24-hour day. No significant differences in discharge rate were observed between these periods of high and low core body temperature either during the entire day (13.2±3.5 versus 11.2±3.0 discharges per hour, respectively $p=0.63$, Figure 16C, D), or during the light or dark phases specifically.
Figure 16

A

B

C

D

---

85
Figure 16. Epileptiform discharge activity in $Mecp2^{+/+}$ mice differs between behavioural states.

Panels A and B: Incidence rate of cortical discharge activity during either the mobile or inactive behavioural states. The histogram (A) shows the mean ± SEM of the discharge rate per hour, normalized to the time spent in each behavioural state as above. Panel B shows a representative plot of epileptiform discharge distribution over the light (i) and dark (ii) phases of a 24-hour day. Red spikes represent individual discharge events and the shaded regions denote times in which mobility was present. Panels C and D: Incidence rate of cortical discharge activity when core body temperature for the $Mecp2^{+/+}$ mice was within the top 25% (high) or the lowest 25% (low) of the mean value for their 24-hour cycle. The histogram (C) shows the mean ± SEM of the discharge rate per hour, normalized for time spent in each temperature category as above. Panel D shows a representative plot in the same similar format as Panels B, except dark shading reflects times when temperature was in the upper 25% and light shading reflects time when temperature was in the lower 25% of the daily range. Times spend in the intermediate temperature range show no shading. Asterisks denote statistical significance ($p<0.05$) as determined using a Student’s paired $t$-test, for n=8 $Mecp2^{+/+}$ mice.
3.2. Targeted Discussion of ‘Daily Rhythmic Behaviours and Thermoregulatory Patterns are Disrupted in Adult Female MeCP2-Deficient Mice’

3.2.1. Daily Cortical Delta Power Patterns are Disrupted in Mecp2+/+ Mice

Although there is clear evidence for disrupted sleep-wake cycles in Rett syndrome patients (Piazza et al., 1990; Ellaway et al., 2001), there have been few assessments of whether normal biological patterning is altered in MeCP2-deficient mice. From this study, our data show that Mecp2+/+ mice display significantly disrupted daily behavioural patterns compared to age and gender-matched wild-type mice. Specifically, Mecp2+/+ mice display reduced numbers of normal cortical delta activity cycles over a 24-hour period. The decrease in number of delta power cycles in Mecp2+/+ was equally prominent in both light and dark phases of the diurnal cycle, indicating that their abnormal cortical delta power patterning is not derived from a deficit in a specific phase. In conjunction with decreased delta power cycle amount, Mecp2+/+ mice also displayed longer average delta power states and consistent with the nocturnal nature of mice, we found a preference for activity in the dark phase of the diurnal cycle as opposed to the light phase.

High delta power has been used as an index for determining sleep and awake times in wild-type animals (Franken et al., 1998; Franken et al., 2001). Consistent with this, we found a strong correlation between periods of high delta power and periods of low activity in wild-type mice. Intriguingly though, this correlation was much weaker and more variant among subjects in Mecp2+/+ mice. Mecp2+/+ mice were observed to have periods of high delta power in addition to high activity. This absence of delta-activity correlation suggests that the normal homeostatic balance of neural circuits is disrupted in the Mecp2+/+ brain as normally, high delta power should not be observed during awake-
mobile states (Franken et al., 1998). These findings seem to be genuine as we have shown that there are no intrinsic fundamental differences in the EEG waveforms between wild-type and MeCP2−/+ mice, which could have confounded our delta power calculations and subsequent correlative strengths. Additionally, we also found similar power spectrum profiles between wild-type and MeCP2−/+ mice. However, we cannot exclude the possibility that a movement artifact caused by the slow ambulatory patterns of MeCP2−/+ mice may have contributed to the delta band signal and thus causing the appearance of high delta power during active states. Irrespective of origin, the clear difference in delta power and activity correlative strength between wild-type and MeCP2−/+ mice illustrates a phenotypic difference that arises from the MeCP2 deficiency.

The observation of disrupted daily rhythmic patterning in MeCP2−/+ mice is consistent with the recent studies that found MeCP2 mRNA to be a direct target of the microRNA miR-132 (Alvarez-Saavedra et al., 2011; Lusardi et al., 2010). miR-132 expression is robustly induced within neurons of the suprachiasmatic nucleus (SCN) by light stimulation (Cheng et al., 2007), and miR-132 negatively regulates MeCP2 protein levels in these neurons. This regulation of MeCP2 expression is one component of the system regulating the expression of Period genes and thus contributes to clock entrainment (Alvarez-Saavedra et al., 2011). Given the strong evidence that disruption of clock gene regulation in SCN is sufficient to alter cortical delta periodicity and power (Franken and Dijk, 2009), the altered delta patterns we observe in MeCP2−/+ mice are in line with MeCP2 playing a significant role in circadian regulation, as suggested by Alvarez-Savaadra et al. (2011). Based on these results and our findings, it would be of
interest to further explore whether alterations of cortical delta activity patterns occur in Rett syndrome patients and/or in patients with other MeCP2-related neural disorders.

3.2.2. *Mecp2*/+ Mice Display Reduced Home-Cage Movement but an Intact Number of Daily Mobility Cycles

In addition to disrupted rhythmic delta cycle patterning, *Mecp2*/+ mice displayed diminished overall movement in their home-cage setting. Consistent with previous results from *Mecp2* male mice (Moretti et al., 2005; De Filippis et al., 2010), the overall amount of activity of female *Mecp2*/+ mice was reduced similarly during the light and dark phases of the diurnal cycle. Similarly, we found the overall number of 30 second segments containing movement was markedly reduced in *Mecp2*/+ mice compared to wild-type controls. The rate of mobility, or movement velocity, was also found to be reduced in *Mecp2*/+ female mice as opposed to wild-type mice and this decrease was most prominent in the dark phase of the diurnal cycle. This is consistent with previous reports of *Mecp2* male mice showing hyperactivity in the form of increased fine movement (Moretti et al., 2005). In contrast to these results, *Mecp2*/+ mice did not display an altered number of mobility cycles over 24 hours compared to wild-type mice. This finding is in support of our observed weak delta-activity parameter correlation in *Mecp2*/+ mice, as number of delta cycles was decreased in *Mecp2*/+ mice.
3.2.3. Thermoregulatory Patterns are Abnormal in Mecp2+/+ Mice

Analysis of the home-cage body temperature profiles of Mecp2+/+ mice revealed alterations in daily temperature cycling patterns in comparison to wild-type mice. Mecp2+/+ mice were found to have significantly fewer transitions between states of low and high body temperature as opposed to wild-type mice and the average duration spent in a particular body temperature state was significantly longer in Mecp2+/+ mice. Additionally, we found that Mecp2+/+ mice showed significant decreases in both their peak minimum and maximum body temperature over the day, and collectively showed an overall decrease in their average body temperature – both throughout the day, and also during periods of activity and inactivity specifically. These observations confirm and build upon those of a recent report in which the basal body temperature of male MeCP2tm1.1Bird mice was found to be reduced compared to wild-type mice (Ward et al., 2011). In addition to showing a decrease in average daily temperatures, our results also show that the range of normal body temperature fluctuation over the day is significantly greater in Mecp2+/+ mice. Finally, we also observed that the correlative strength between temperature and activity parameters in Mecp2+/+ mice is significantly weaker than wild-type mice. All together these results offer strong evidence that Mecp2+/+ mice possess dysfunctional thermoregulatory control that results in their inability to maintain a proper body temperature homeostasis. Collectively, these results are consistent with impaired autonomic nervous system function, which is a cardinal phenotype of clinical Rett syndrome.
3.2.4. Mecp2+/Mice Display Epileptiform Discharges that Predominate During Activity

In agreement with the previous acute study from our laboratory (D’Cruz et al., 2010), we observed the presence of abnormal epileptiform-like discharges in the somatosensory cortex of Mecp2+/mice. As with the previous study from our laboratory, no epileptiform discharge events were observed in wild-type mice and discharges were found in the majority, but not all of the Mecp2+/mice. The absence of epileptiform discharges in some of the animals despite being over 300 days old indicates that their lack of discharges is not due to them being too young and that these animals possess a non-epileptic phenotype. Our characterization of these discharge events revealed no significant differences in their average incidence, duration, and frequency component between light and dark phases of the diurnal cycle. However, the incidence rate for discharge activity did correlate with times when the mutant mice were in specific behavioural states. Significantly more discharge activity was observed in the mutants during times of activity and/or movement compared to times of immobility. Surprisingly however, no differences in discharge rate were seen in the mice when their body temperature was in the upper or lower 25% of their daily range. This result was somewhat unexpected, as lower temperature tends to slow metabolic processes, and has been linked to an attenuation of seizure rates (Rothman et al., 2005). The most likely explanation for this is that although lower, the decreased core body temperature is not sufficient to have a major effect on neural activity, and thus the hyper-excitability of the MeCP2-deficient circuits is not diminished.
3.3. Correlation of Regional MeCP2 Expression Levels in the Female MeCP2-Deficient Mouse Brain with Specific Behavioural Impairments\textsuperscript{4}

3.3.1. MeCP2 Expression Levels Differ Significantly Between Cortex, Hippocampus, Cerebellum, and Spinal Cord within Individual Adult Mecp2\textsuperscript{+/+} Mice

Immunoblot analysis of Mecp2\textsuperscript{+/+} cortex, cerebellum, hippocampus, and spinal cord revealed MeCP2 protein levels in these structures ranged from between 18-75% that of wild-type levels across the cohort (Mecp2\textsuperscript{+/+} n=10 and WT n=4, Figure 17). In addition to displaying a wide range of expression between subjects, MeCP2 expression levels also varied between regions of the same Mecp2\textsuperscript{+/+} mouse (Figure 18A-D). The variance in expression levels between regions was occasionally quite large, as mice having the highest relative MeCP2 protein level in one region could display the lowest relative MeCP2 protein level in another region (see mouse 9 and mouse 10 of the cohort in Figure 18 for examples). In fact, Pearson’s product-moment analysis demonstrated the expression levels of MeCP2 in one brain region did not significantly correlate with its expression levels in another brain region (Table 2). The only exception to this was that the cerebellum was found to correlate strongly with MeCP2 amounts in the spinal cord (r=0.937, \( p < 0.001 \), Table 2). Despite the correlation between the cerebellum and the spinal cord, these results seem to indicate that MeCP2 is not uniformly expressed across the different structures of the Mecp2\textsuperscript{+/+} mouse brain, and that the level of MeCP2 in one region does not necessarily predict its level of expression within another region.

\textsuperscript{4} Some aspects of this results chapter have been published in: Wither, R.G., Lang, M., Zhang, L., Eubanks, J.H., 2012. Regional MeCP2 expression levels in the female MeCP2-deficient mouse brain correlate with specific behavioral impairments. Exp Neurol. \textit{In Press}.
Figure 17

A
i) Wild-type
MeCP2

ii) GAPDH

B
i) Mecp2"''
MeCP2

ii) GAPDH

C

Normalized MeCP2 Levels (%) vs. Mouse

0 20 40 60 80 100
1 2 3 4 5 6 7 8 9 10

Mouse
Figure 17. Whole brain MeCP2 protein levels in Mecp2<sup>+/+</sup> mice range between 18-75% compared to wild-type MeCP2 levels.

Panels A-B: Western blot results showing the immunoreactive levels of MeCP2 (i) in whole brain tissue samples from wild-type (A) and Mecp2<sup>+/+</sup> (B) mice. The immunoreactive levels of GAPDH (ii) from the same blot are shown below to serve as a control for protein loading. Panel C: Scatter plot showing the relative GAPDH-normalized densitometric intensity ratio for MeCP2 in each of the Mecp2<sup>+/+</sup> mice expressed as a percentage of wild-type (denoted as 100% on the y axis). In this scatter plot, Mecp2<sup>tm1.1Bird</sup> mice are represented by circular symbols while Mecp2<sup>tm2Bird</sup> mice are represented by triangular symbols.
Figure 18

A

i) MeCP2

ii) GAPDH

Cortex

iii) Normalized MeCP2 Levels (%)

Mouse

B

i) Cerebellum

ii) Normalized MeCP2 Levels (%)

Mouse

C

i) Hippocampus

ii) GAPDH

MeCP2

iii) Normalized MeCP2 Levels (%)

Mouse

D

i) Spinal Cord

ii) Normalized MeCP2 Levels (%)

Mouse
Figure 18. MeCP2 protein levels vary among Mecp2<sup>+</sup> mice and brain region.

Panels A-D: Representative Immunoblot results showing the amount of MeCP2 (i), GAPDH (ii), and the average of the triplicate densitometric measurements of MeCP2 protein (iii) in the cortex (A, n=10), cerebellum (B, n=10), hippocampus (C, n=9), and spinal cord (D, n=8) tissue homogenates of Mecp2<sup>+</sup> mice across. In the scatter plots, GAPDH protein levels were used to normalize for protein loading levels across different Mecp2<sup>+</sup> mice and within each blot, each mouse is normalized to the MeCP2 value of mouse 7. The error bars indicate the SEM of the data. Mecp<sup>tm1.1Bird</sup> mice are represented by circular symbols while Mecp<sup>tm2Bird</sup> mice are represented by triangular symbols.
Table 3. Pearson correlation coefficients comparing specific brain region MeCP2 protein levels in Mecp2<sup>+/−</sup> mice.

Summary table of correlation coefficients (linear regression ‘r’ values) and statistical p values for the comparisons of cortical, hippocampal, cerebellar, and spinal cord MeCP2 protein levels in MeCP2<sup>+/−</sup> mice.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Cortex</th>
<th>Cerebellum</th>
<th>Hippocampus</th>
<th>Spinal Cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.530, 0.11</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.518, 0.16</td>
<td>0.329, 0.85</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>0.490, 0.22</td>
<td><strong>0.937, 0.001</strong></td>
<td>0.242, 0.60</td>
<td>N/A</td>
</tr>
</tbody>
</table>
3.3.2. Cortical MeCP2 Levels Display an Inverse Correlation with the Overall Phenotypic Severity of Female Mecp2+/− Mice

Having shown differences in MeCP2 expression levels in different brain regions of MeCP2-deficient mice, we then tested whether the levels of MeCP2 protein in a specific brain region would correlate with the phenotypic severity and/or degree of impairment the individual mice displayed in specific behavioural tests. To assess phenotypic severity, we applied the 12-point gross phenotype severity scale previously described (Guy et al., 2007) to individual Mecp2+/− mice of 300-400 days of age. Using this scale, our cohort of mice displayed affectedness scores ranging from 2 to 9 (Figure 19). Pearson’s product-moment analysis of these scores with the relative MeCP2 protein levels in hippocampus, cerebellum, or spinal cord failed to identify any significant correlations (Figure 19, Table 3). Analysis of cortex, however, revealed a strong inverse correlation between cortical MeCP2 protein levels and the overall phenotypic severity score assigned to the individual Mecp2+/− mice (r=−0.798, p<0.05, Figure 19A).
Figure 19

A  Cortex

B  Cerebellum

C  Hippocampus

D  Spinal Cord

Symptom Severity Score vs. Normalized MeCP2 Levels (%) for each region.
Figure 19. Overall phenotypic symptom severity correlates with cortical MeCP2 protein levels in \textit{Mecp2}^{+/+} mice.

Panel A-D: Scatter plots comparing cortical (A, \(n=10\)), cerebellar (B, \(n=10\)), hippocampal (C, \(n=9\)), and spinal cord (D, \(n=8\)) MeCP2 protein levels against overall symptom severity scores in \textit{Mecp2}^{+/+} mice as determined using the Bird 12-point scale. In all scatter plots the line represents the linear regression of the data set whereas the dotted lines represent the 95\% confidence interval of the linear regression. \textit{Mecp2}^{tm1.1Bird} mice are represented by circular symbols while \textit{Mecp2}^{tm2Bird} mice are represented by triangular symbols.
Table 4. Pearson correlation coefficients comparing regional MeCP2 protein levels to specific behavioural performance parameters.

Summary table of correlation coefficients (linear regression ‘r’ values) comparing cortical, hippocampal, cerebellar, and spinal cord MeCP2 protein levels in Mecp2<sup>−/−</sup> mice against their overall phenotypic severity, total activity, total rearing, number of risk assessing head pokes, time spent risk assessing, center rearing, number of daily delta cycles, epileptiform discharge rates, and average and minimum body temperatures. Bolded and italicized text specifies correlations displaying statistical significance (<i>p</i>&lt;0.05).

<table>
<thead>
<tr>
<th>Behavior Test</th>
<th>Cortex (n=10)</th>
<th>Cerebellum (n=10)</th>
<th>Hippocampus (n=9)</th>
<th>Spinal Cord (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic Severity</td>
<td>-0.798</td>
<td>-0.466</td>
<td>-0.506</td>
<td>-0.231</td>
</tr>
<tr>
<td>Total Activity</td>
<td>0.651</td>
<td>0.387</td>
<td>0.785</td>
<td>0.358</td>
</tr>
<tr>
<td>Total Rearing</td>
<td>0.444</td>
<td>0.437</td>
<td>0.870</td>
<td>0.353</td>
</tr>
<tr>
<td>Risk Assessments</td>
<td>0.379</td>
<td>0.079</td>
<td>0.823</td>
<td>0.031</td>
</tr>
<tr>
<td>Risk Assessment Duration</td>
<td>0.398</td>
<td>0.166</td>
<td>0.821</td>
<td>0.255</td>
</tr>
<tr>
<td>Center Rearing</td>
<td>0.486</td>
<td>0.547</td>
<td>0.919</td>
<td>0.519</td>
</tr>
<tr>
<td>Daily Delta Cycles</td>
<td>0.747</td>
<td>0.858</td>
<td>0.320</td>
<td>0.749</td>
</tr>
<tr>
<td>Discharge Rate</td>
<td>-0.301</td>
<td>-0.443</td>
<td>-0.770</td>
<td>-0.129</td>
</tr>
<tr>
<td>Average Temperature</td>
<td>0.266</td>
<td>0.418</td>
<td>0.192</td>
<td>0.358</td>
</tr>
<tr>
<td>Minimum Temperature</td>
<td>0.369</td>
<td>0.516</td>
<td>0.229</td>
<td>0.400</td>
</tr>
</tbody>
</table>
3.3.3. Hippocampal MeCP2 Levels Display Positive Correlations with the Activity and Rearing Behaviour of Female Mecp2+/− Mice

Pearson’s product-moment analysis revealed significant positive correlations between hippocampal MeCP2 levels and the total activity scores of individual Mecp2+/− mice in the open field (r=0.785, p<0.05, Figure 20C). No significant correlations were seen between total activity and MeCP2 levels in cortex, cerebellum, or spinal cord (Figure 20). In addition to correlating with total activity, hippocampal MeCP2 levels also correlated significantly with the rearing activity of individual Mecp2+/− mice (r=0.870, p<0.05, Figure 20G). No significant correlations were observed between MeCP2 levels in cortex, cerebellum, or spinal cord with the rearing activity of Mecp2+/− mice in the open field (Figure 20, Table 3).
Figure 20

A  Cortex

B  Cerebellum

C  Hippocampus

D  Spinal Cord

Total Activity Beam Breaks vs. Normalized MeCP2 Levels (%)
Figure 20 Cont’d

E. Cortex

F. Cerebellum

G. Hippocampus

H. Spinal Cord

Graphs showing the relationship between normalized MeCP2 levels and total rearing beam breaks for different brain regions.
Figure 20. Hippocampal MeCP2 protein levels correlate with total activity and rearing parameters of Mecp2+/+ mice in an open field behavioural test.

Panels A-D: Scatter plots comparing the total amount of activity Mecp2+/+ mice exhibited in an open field apparatus against cortical (A, n=9), cerebellar (B, n=9), hippocampal (C, n=8), and spinal cord (D, n=7) MeCP2 protein levels. Panels E-H: Scatter plots comparing total rearing of Mecp2+/+ in an open field apparatus against cortical (E, n=9), cerebellar (F, n=9), hippocampal (G, n=8), and spinal cord (H, n=7) MeCP2 protein levels. In all scatter plots the line represents the linear regression of the data set whereas the dotted lines represent the 95% confidence interval of the linear regression. Mecp2tm1.1Bird mice are represented by circular symbols while Mecp2tm2Bird mice are represented by triangular symbols.
3.3.4. MeCP2 Levels in the Hippocampus Correlate with Anxiety-like Behaviour in Female MeCP2<sup>−/+</sup> Mice

The anxiety-like behaviour of MeCP2<sup>−/+</sup> mice was assessed using the light/dark place preference test and also by examining the center area rearing activity of the mice in the open field test. In the light/dark place preference test, both the number of risk assessments from the dark compartment to the light compartment, and the total time of risk assessment, correlated strongly with hippocampal MeCP2 levels (r=0.823 and r=0.821 respectively, p<0.01 for each, Figure 21C, G). Similarly, a strong correlation was observed between hippocampal MeCP2 levels and the rearing activity of the mice in the center area of the open field apparatus (r=0.919, p<0.01, Figure 22C). In contrast, Pearson’s product-moment analysis of MeCP2 levels in cortex, cerebellum, and spinal cord failed to identify any significant correlations with either of these anxiety-like behaviours (Figures 21 and 22, Table 3).
Figure 21

A  Cortex

B  Cerebellum

C  Hippocampus

D  Spinal Cord

Risk Assessments vs. Normalized MeCP2 Levels (%)
Figure 21 Cont’d

E  Cortex

F  Cerebellum

G  Hippocampus

H  Spinal Cord
Figure 21. Extent of MeCP2 protein deficiency in the hippocampus of *Mecp2*\(^{+/+}\) mice significantly correlate with risk taking behaviour.

Panels A-D: Scatter plots comparing the amount of risk-assessment head pokes performed by *Mecp2*\(^{+/+}\) mice against protein levels of MeCP2 in the cortex (A, n=10), cerebellum (B, n=10), hippocampus (C, n=9), and spinal cord (D, n=8). Panels E-H: Scatter plots showing the duration of risk-assessing behaviour against functional MeCP2 protein levels in the cortex (E, n=10), cerebellum (F, n=10), hippocampus (G, n=9), and spinal cord (H, n=8) from *Mecp2*\(^{+/+}\) mice. In all scatter plots the line represents the linear regression of the data set whereas the dotted lines represent the 95% confidence interval of the linear regression. *Mecp2*\(^{tm1.1Bird}\) mice are represented by circular symbols while *Mecp2*\(^{tm2Bird}\) mice are represented by triangular symbols.
Figure 22

A: Cortex

B: Cerebellum

C: Hippocampus

D: Spinal Cord
Figure 22. Center rearing behaviour correlates significantly with hippocampal MeCP2 protein values in $MeCP2^{+/+}$ mice.

Panels A-D: Scatter plots comparing the total amount of center rearing behaviour exhibited by $MeCP2^{+/+}$ mice in an open field arena against the amount of functional MeCP2 in the cortex (A, n=9), cerebellum (B, n=9), hippocampus (C, n=8), and spinal cord (D, n=7). In all scatter plots the line represents the linear regression of the data set whereas the dotted lines represent the 95% confidence interval of the linear regression. $MeCP2^{tm1.1Bird}$ mice are represented by circular symbols while $MeCP2^{tm2Bird}$ mice are represented by triangular symbols.
3.3.5. MeCP2 Levels in Cortex, Cerebellum, and Spinal Cord Correlate with the Number of Daily Delta Power Cycles in Female Mecp2+/+ Mice

Mice normally display rhythmic patterns of cortical EEG delta power over a 24-hour cycle, alternating between periods of high and low delta power. As we have observed that these delta power patterns on average are altered in female MeCP2-deficient mice, we therefore tested whether the degree of rhythmic delta power alteration would correlate with MeCP2 expression levels in cortex, or other brain regions. As anticipated, cortical MeCP2 expression levels displayed a significant positive correlation with the number of daily EEG delta power cycles in Mecp2+/+ mice (r=0.747, p<0.05, Figure 23A). Surprisingly, a strong positive correlation was also seen for MeCP2 expression levels in cerebellum and spinal cord (r=0.858 and r=0.749 respectively, p<0.05 for both, Figure 23B, D). No significant correlations were observed for hippocampal MeCP2 levels and the number of delta power cycles (Figure 23).
Figure 23

A  Cortex

B  Cerebellum

C  Hippocampus

D  Spinal Cord

Daily Delta Cycles

Normalized MeCP2 Levels (%)
Figure 23. Cortical, cerebellar and spinal cord MeCP2 protein amounts correlate with the degree of daily rhythmic delta cycling impairment in $Mecp2^{+/+}$ mice.

Panels A-D: Scatter plots of the number of delta cycles over 24 hours exhibited by the $Mecp2^{+/+}$ mice against functional cortical (A, $n=10$), cerebellar (B, $n=10$), hippocampal (C, $n=9$), and spinal cord (D, $n=8$) MeCP2 protein levels. In all scatter plots the line represents the linear regression of the data set whereas the dotted lines represent the 95% confidence interval of the linear regression. $Mecp2^{tm1.1Bird}$ mice are represented by circular symbols while $Mecp2^{tm2Bird}$ mice are represented by triangular symbols.
3.3.6. MeCP2 Levels in Hippocampus Display an Inverse Correlation with Epileptiform Discharge Activity in Female Mecp2+/− Mice

Epilepsy is a common co-morbidity of clinical Rett syndrome and as we have shown previously female Mecp2+/− mice display a range of epileptiform discharge activities in their cortex (D’Cruz et al., 2010). Analysis of the number of discharges observed over a 24-hour day with the level of MeCP2 protein in hippocampus revealed a strong inverse correlation (r=−0.770, p<0.05, Figure 24C). Surprisingly, however, no correlation was observed between cortical MeCP2 protein levels and epileptiform discharge activity (Figure 24A, Table 3). Not surprisingly, no significant correlations were found between discharge activity and either cerebellum or spinal cord MeCP2 protein levels (Figure 24, Table 3).
Figure 24

A. Cortex

B. Cerebellum

C. Hippocampus

D. Spinal Cord

Normalized MeCP2 Levels (%) vs. Discharges per Hour
Figure 24. MeCP2 protein levels in the hippocampus of \( Mecp2^{+/+} \) mice significantly correlate with incidence of epileptiform discharges displayed over 24 hours.

Panels A-D: Scatter plots comparing the incidence of epileptiform discharges displayed by \( Mecp2^{+/+} \) mice over 24 hours against the functional MeCP2 protein levels in the cortex (A, n=10), cerebellum (B, n=10), hippocampus (C, n=9), and spinal cord (D, n=8). In all scatter plots the line represents the linear regression of the data set whereas the dotted lines represent the 95% confidence interval of the linear regression. \( Mecp2^{tm1.1Bird} \) mice are represented by circular symbols while \( Mecp2^{tm2Bird} \) mice are represented by triangular symbols.
3.3.7. *MeCP2 Levels in Cortex, Hippocampus, Cerebellum and Spinal Cord do not Correlate with Diminished Body Temperature Level in Female *Mecp2*+/−* Mice*

Autonomic nervous system impairment is commonly observed in Rett syndrome (Julu et al., 2001; Glaze, 2005), and our previous results demonstrated that the average body temperature and the minimum temperature over a 24-hour day are diminished in *Mecp2*+/− mice. We therefore tested whether MeCP2 expression levels in cortex, hippocampus, cerebellum or spinal cord would correlate with the magnitude of daily thermoregulatory alterations in female *Mecp2*+/− mice. In contrast to the significant correlations seen between regional MeCP2 levels and behavioural impairments above, Pearson’s product-moment analysis failed to identify significant correlations between MeCP2 protein levels in any of the tested regions and either average body temperature, or the absolute minimal body temperature displayed by *Mecp2*+/− mice over the 24-hour day (*p*>0.05, Figure 25). This is expected as the hypothalamus, not the brain regions examined, is the critical brain structure for the regulation of core body temperature.
Figure 25

A. Cortex

B. Cerebellum

C. Hippocampus

D. Spinal Cord

Temperature (°C)

Normalized MeCP2 Levels (%)
**Figure 25.** Impaired body temperature profiles of *Mecp2*<sup>+/−</sup> mice do not correlate with MeCP2 protein levels in the cortex, cerebellum, hippocampus, or spinal cord.

Panels A-D: Scatter plots of the average body temperature of *Mecp2*<sup>+/−</sup> mice over 24 hours compared against MeCP2 protein levels in the cortex (A, n=9), cerebellum (B, n=9), hippocampus (C, n=8), and spinal cord (D, n=7). Panels E-H: Scatter plots comparing the absolute minimum body temperature exhibited by *Mecp2*<sup>+/−</sup> mice during 24 hours against MeCP2 protein amounts in the cortex (E, n=9), cerebellum (F, n=9), hippocampus (G, n=8), and spinal cord (H, n=7). In all scatter plots the line represents the linear regression of the data set whereas the dotted lines represent the 95% confidence interval of the linear regression. *Mecp2<sup>tm1.1Bird</sup>* mice are represented by circular symbols while *Mecp2<sup>tm2Bird</sup>* mice are represented by triangular symbols.
3.4. Targeted Discussion of ‘Correlation of Regional MeCP2 Expression Levels in the Female MeCP2-Deficient Mouse Brain with Specific Behavioural Impairments’

3.4.1. MeCP2 Expression Levels do not Correlate Between Different Brain Regions in Mecp2\textsuperscript{+/+} Mice

In this study, we tested whether or not the amount of MeCP2 protein expressed in adult female Mecp\textsubscript{2}\textsuperscript{-/-} mice would be consistent, or vary significantly between different neural regions. We found that no level of MeCP2 in one brain region predicted the level in another region except for the cerebellum and spinal cord regions. The observed correlation between cerebellar and spinal cord MeCP2 levels may be due to the fact the cerebellum and spinal cord are derived from similar progenitors and that X-chromosome inactivation patterns are different during different stages of embryogenesis as well as germ cell layers (Heard and Disteche, 2006; Park et al., 2007). This observed correlation between the cerebellum and the spinal cord does not detract from the conclusion that our results, overall, show that the levels of expressed MeCP2 protein can vary dramatically between different neural tissues within individual subjects. This result was somewhat unexpected, as a previous study found no evidence for differential skewing of XCI patterns between catecholaminergic neurons in the paraventricular hypothalamic nucleus and parvalbumin-positive cells in the cerebral cortex in a related Mecp\textsubscript{2}\textsuperscript{308} male mouse model (Young and Zoghbi, 2004). The reason for the seemingly different outcomes is not clear, but could relate to the different mouse models employed, or the different central nervous system regions examined. We did not assess XCI specifically in this study, and thus, it is unclear whether the difference in MeCP2 protein levels we detect within these structures arises from different ratios of XCI in these tissues. In addition to XCI skewing,
MeCP2 expression levels can be influenced by micro-RNA regulation of Mecp2 transcript levels (Klein et al., 2007; Alvarez-Saavedra et al., 2011), post-translational MeCP2 protein modifications (Zhou et al., 2006; Rexach et al., 2010), and the wild-type MeCP2 allele expression can be influenced by its surrounding environment through non-cell autonomous mechanisms (Brauchensweig et al., 2004). Collectively, each of these phenomena could contribute to the MeCP2 levels observed in each tissue. Despite the unclear mechanisms, however, our results provide clear evidence that MeCP2 protein level can vary dramatically between central nervous system regions within individual subjects, and as such, suggest that different regions of the brain could possess differing levels of functionality within a single subject. Thus, in conjunction with ubiquitously skewed XCI, these results provide an additional mechanism through which the phenotypic variance observed in female MeCP2-deficient mice could arise.

3.4.2. Overall Symptom Severity Correlates with Cortical but not Spinal Cord MeCP2 Levels in Mecp2+/− Mice

In addition to showing regional differences in MeCP2 expression, we also tested whether or not the level of expression correlated with the degree of overall behavioural or neurophysiological deficit present in these Mecp2+/− mice. Our results show that there is a significant negative correlation observed between cortical MeCP2 amounts and overall gross symptom severity. This result is not unexpected as selectively ablating MeCP2 from forebrain neurons is sufficient to induce several Rett-like behavioural impairments in mice (Chen et al., 2001; Gemelli et al., 2006) and the 12-point severity scale we employed scores behavioural features that have at least some dependence on cortical...
function (e.g., inertia, gait, hind-limb clasp, and general presentation) (Guy et al., 2007). What was perhaps surprising was the lack of any correlation between spinal cord MeCP2 levels with the overall symptom severity scores, given the likely role of this structure in these general features. The reason for this lack of correlation is unclear, but may suggest spinal cord function has a tolerance for partial MeCP2 deficiency, and/or that spinal cord activity in MeCP2-deficient mice is over-ridden by the functionality of projecting circuits that stem from other areas of the brain.

3.4.3. Anxiety-like Behaviour and Exploratory Impairments Correlate with Hippocampal MeCP2 Expression Levels in Mecp2+/− Mice

We also identified correlations between regional MeCP2 expression levels and specific phenotypic impairments in female MeCP2-deficient mice. The rearing and total activity in the open field test, as well as specific anxiety-like behaviours of Mecp2+/− female mice displayed a positive correlation with hippocampal MeCP2 protein levels. These correlations between exploratory and risk assessing behaviours with hippocampal MeCP2 protein levels is not surprising as the hippocampus is critical in regulating anxiety-like behaviours (Barkus et al., 2010; McHugh et al., 2004) and serotonergic innervation is disrupted in MeCP2-deficient mice (Isoda et al. 2010; Ren-Patterson et al. 2005). It should be noted though, that the amygdala was included in the hippocampal tissue sample analyzed and this may have contributed to the observed correlation between hippocampal MeCP2 protein levels and anxiety-like behaviours as the amygdala is the primary structure involved in the expression of fear and anxiety-like behaviour.
The lack of a correlation between locomotive parameters and cerebellar MeCP2 levels is not entirely unexpected either, as it has been shown that severe cerebellar damage does not result in noticeable deficits in the open field activity amounts of rats (Mandolesi et al., 2010; Molinari et al., 1990). One surprising finding was the lack of a cortical correlation with exploratory behaviour as the cortex regulates voluntary movement and the motor cortex of MeCP2-null mice has been shown to be morphologically abnormal. In the absence of MeCP2 there is reduced dendritic spine density, complexity, length and size, as well as abnormal axonal organization and soma size of pyramidal neurons in the motor cortex (Belichenko et al., 2009; Robinson et al., 2012). Also of interest is the lack of a correlation between total activity behaviour and spinal cord MeCP2 protein levels in the MeCP2\(^{-/-}\) mice, further suggesting that spinal cord function possesses a tolerance to partial MeCP2-deficiency.

3.4.4. Hippocampal MeCP2 Protein Levels Correlate with Epileptiform Discharge Incidence in MeCP2\(^{-/+}\) Mice

In this study we also examined whether regional MeCP2 expression correlated with the incidence rate of epileptiform discharges. At the network level, we expected discharge activity would correlate with cortical MeCP2 levels given that previous results from our lab suggest an absence-like nature of the EEG activity (D’Cruz et al., 2010) and absence seizures are known to originate in thalamo-cortical networks (Crunelli and Leresche, 2002; McCormick and Contreras, 2001). Thus, the lack of an observed significant correlation between cortical MeCP2 levels and discharge activity was surprising. The inverse correlation seen between hippocampal MeCP2 levels and
discharge activity is intriguing, but not necessarily unexpected, as previous reports have shown MeCP2-deficient hippocampal networks are hyper-exitable (Zhang et al., 2008; Calfa et al., 2011). The role of the hippocampus in the generation of in vivo discharge activity in MeCP2-deficient mice remains largely unknown, and our current results suggest that examining its role in this activity warrants further examination.

3.4.5. Cortical, Cerebellar, as well as Spinal Cord MeCP2 Levels Correlate with Number of Daily Delta Cycles in Mecp2<sup>+/−</sup> Mice

Since we had observed that Mecp2<sup>+/−</sup> female mice displayed reduced levels of daily cortical delta power cycles we tested whether the regional MeCP2 expression correlated with the degree of impairment in this patterning. We observed a positive correlation between cortical, cerebellar, and spinal cord MeCP2 levels and the number of daily cortical delta frequency band cycles. The correlation between delta cycle impairment and cortical MeCP2 levels is not very surprising as high delta power is known to be indicative of a non-REM sleep state (Franken et al., 1998; Dang-Vu et al., 2005) and the cortex is necessary for the expression of non-REM sleep (de Andrés et al., 2011). The correlation found between cerebellar MeCP2 levels and cortical delta power cycles is also not entirely unexpected as there has been a study showing that the cerebellum is involved with sleep regulation and modulation (Paz et al., 1982). The correlation between spinal cord and delta cycles is very unexpected as the spinal cord is not known to be involved in regulating sleep. An explanation for this observed correlation is that the spinal cord does have similar MeCP2 expression as the cerebellum,
which, as just mentioned, is involved in sleep regulation. Therefore this correlation could be spurious due to the similar MeCP2 expressions in the cerebellum and spinal cord.

3.4.6. The Abnormal Thermoregulatory Patterns of Mecp2+/− Mice did not Correlate with any of the Region-Specific MeCP2 Levels Examined

Finally, we observed no correlations between MeCP2 levels in any of the regions examined and the extent of thermoregulatory impairment in Mecp2+/− mice. This lack of correlation was expected, as thermoregulatory patterns are largely influenced by hypothalamic neurons (Refinetti and Carlisle, 1986; Hellon and Taylor, 1982), which were not specifically examined in our study. While thermoregulation impairments have not been examined in mice with forebrain targeted MeCP2 ablation, our observation is consistent with the results of Ward et al. (2011), who showed selective ablation of MeCP2 from HoxB1-expressing hindbrain and spinal cord neurons, which excludes the hypothalamus, failed to alter thermoregulatory patterns in mice.
Chapter 4

Discussion

4.1. General Discussion of Project

4.1.1. Project Summary

Although RTT girls typically present with disrupted daily sleep and behavioural patterns as well as hypothermia, there has been remarkably little focus on these deficits and whether MeCP2-deficient mice recapitulate them. In the first part of this study, we examined the daily periodic cortical EEG waveform activity, body temperature, and movement activity parameters of MeCP2 mice in their home-cage setting. Five principal observations emerge from our work. First, the normal daily pattern of cyclic EEG delta frequency band activity is altered in MeCP2 mice, specifically these mutants display a decreased number of daily delta cycles, and spend longer periods than normal in a low delta power state. Second, MeCP2 mice display significantly less movement in their home-cage environment, particularly during the nocturnal phase, and display significantly more time in an awake-but-inactive state. Third, the daily minimum, maximum, and overall average temperature of MeCP2 mice is lower than that of wild-type mice. Fourth, MeCP2 mice display spontaneous cortical epileptiform discharges, and this discharge activity is most pronounced when the mouse is in an active behavioural state. Fifth, the daily rhythmic and correlative patterns of delta power, movement activity, and body temperature are significantly altered in MeCP2 mice. Collectively, these investigations identify novel behavioural deficits associated with
MeCP2 deficiency, and provide a new investigative procedure that can be employed for further studies in assessing circadian impairments.

In the second part of this study, we examined the precise relationship between MeCP2 protein expression in specific central nervous system regions and the extent of behavioural and neurological deficits exhibited by MeCP2−/+ mice. We built upon the findings in the first part of this study by identifying a strong correlational link between MeCP2 protein levels in the cortex, cerebellum, and spinal cord of MeCP2−/+ mouse brains and the number of daily cortical delta cycles observed over 24 hours. Additionally, we found that hippocampal MeCP2 protein levels inversely correlated with the amount of epileptiform discharges exhibited over 24 hours by MeCP2−/+ mice. We also examined whether the thermoregulatory deficits of these mice could be explained by MeCP2 deficits in the cortex, cerebellum, hippocampus, or spinal cord, but failed to find a significant correlation. Further correlations between hippocampal MeCP2 protein levels and exploratory and anxiety-like behaviours, were also noted in MeCP2−/+ mice. Finally, we also noted a lack of correlation between the MeCP2 protein amounts in all of the brain regions examined except between the cerebellum and spinal cord, suggesting that MeCP2 expression is not completely uniform across the MeCP2−/+ mouse brain. Collectively these results indicate that behavioural deficit severity is dependent on specific regional MeCP2 protein levels and offers an additional explanation for the wide variety of RTT phenotypes observed clinically.
4.1.2. Non-uniform Regional MeCP2 Expression Offers an Explanation for RTT Phenotypic Variance

The results from this study show the MeCP2 protein expression levels in one region of the brain do not mirror expression levels in other regions. This finding, coupled with our finding that MeCP2 protein expression in a specific brain region can correlate with behavioural deficit severity, suggests the possibility of phenotypes in which some behaviours may be very severely impaired while others remain relatively intact. In fact, the mice examined in this study illustrate this possibility, as some of the mice had very variable phenotypic presentations. For example, we observed mice which displayed widespread behavioural impairments in all categories assessed but failed to produce a single epileptiform discharge, as well as mice which seemed relatively wild-type in all aspects with the exception of extremely heightened anxiety-like behaviour. This is consistent with what has been seen clinically in RTT girls as well. Some girls with a diagnosis of typical RTT do not ever display epileptic seizures (Glaze et al., 2010), as well as only some individuals develop and manifest a phenotype of peripheral hypothermia (Naidu et al., 1987). Other studies have also arrived at similar conclusions, where deficits in some behaviours do not necessitate deficits in others (Nielsen et al., 2001; Hoffbuhr et al., 2002).

Whether this non-uniform MeCP2 protein expression in the MeCP2 female mouse brain is directly related to non-uniform XCI patterns between regions remains to be elucidated. As it stands, we do not precisely know if the correlation we observe between MeCP2 protein level and degree of behavioural impairment is a consequence of fewer/more cells expressing MeCP2 or the same proportion of cells expressing more or
less MeCP2 protein. The most simplistic, elegant answer would be that non-random preferential skewing of the MeCP2 allele through XCI underlies these MeCP2 protein expression patterns and correlations, and there is considerable evidence for this, as XCI patterns do correlate with phenotypic severity (Amir et al., 2000; Young and Zoghbi, 2004). The alternative explanation, that these correlations are derived from the same proportion of wild-type MeCP2 allele cells expressing differing MeCP2 protein amounts in different MeCP2+/ mice is not nearly as compelling as the former, but there is evidence supporting this possibility. There have been studies that have failed to find a correlation between XCI patterning and phenotypic severity in RTT patients (Nielsen et al., 2001; Hoffbuhr et al., 2001), suggesting that factors outside of XCI may control MeCP2 expression levels enough to manifest distinct phenotypes. This is consistent with other studies which have shown that MeCP2 levels are under non-autonomous control at a cellular level and that miR-132, which is inducible through activity, represses MeCP2 protein expression (Alvarez-Saavedra et al., 2011; Braunschweig et al., 2004). Most likely, MeCP2 expression is under complex control, depending on XCI patterns and a multitude of molecular factors. It would therefore be of significant interest to determine to what extent XCI patterns contribute to skewed MeCP2 protein expression levels.

Regardless of the mechanisms underlying MeCP2 protein expression, these results seem to support the possibility that partial restoration of MeCP2 to specific regions of the MeCP2-deficient brain may improve behavioural deficits associated with the compromised function of that region. This is an important finding with regards to the prospect of potentially using gene therapy as a method of intervention for RTT patients. Systemic delivery remains the biggest challenge to overcome for successfully
implementing gene therapy, so the knowledge that increased regional MeCP2 levels correlates with better behavioural performance provides a basis for the use of targeted localized gene therapy. Local delivery also provides the added benefits of avoiding immune detection and uptake, reducing systemic toxicity, and assisting in successful delivery to target cells (Zhang et al., 2012).

4.1.3. Evidence for Atypical Absence Seizures in MeCP2\(^{-/+}\) Female Mice

Absence seizures are characterized by the occurrence of generalized spike and wave discharges (SWDs) at a frequency of 7 to 9 Hz in EEG waveforms (Cortez et al., 2001; Jando et al., 2005). Specifically, SWDs in the thalamo-cortical networks are indicative of typical absence seizures (Banerjee et al., 1993), whereas SWDs involving hippocampal as well as thalamocortical circuitry is indicative of atypical absence seizures (Cortez et al., 2001). In this study, as well as a previous study by our laboratory we observed SWD events in MeCP2\(^{-/+}\) mice similar to those seen in other rodent models of absence epilepsy, suggesting that the discharges in MeCP2\(^{-/+}\) mice are absence in nature (D’Cruz et al., 2010). Consistent with this, the discharges in MeCP2\(^{-/+}\) mice have been found to be susceptible to ethosuximide, a primarily anti-atypical absence pharmaceutical, and are accompanied by behavioural arrest (D’Cruz et al., 2010).

Our findings that these absence-like epileptiform discharges correlated with MeCP2 protein amounts in the hippocampus, may suggest that hippocampal circuitry is involved in the genesis of these discharges. Since atypical absence seizures are characterized by limbic involvement, it therefore stands to reason that these discharges observed in the MeCP2\(^{-/+}\) mice may be indicative of atypical absence seizures.
Additionally, it has been noted that atypical absence seizures in mice can be responsive to ethosuximide (Wu et al., 2007) similar to what we observe with the discharges in Mecp2<sup>+/</sup> mice (D’Cruz et al., 2010). However, the previous work by our laboratory noted that there was no evidence of hippocampal SWDs in Mecp2<sup>+/</sup> mice (D’Cruz et al., 2010), as well as the SWDs associated with atypical absence seizures in mice are usually slow, with a frequency between 4-6 Hz, whereas those we observed are between 7-9 Hz (Wang et al., 2009). Additionally, atypical absence seizures are generally significantly longer than the average discharge duration we reported in this study and are not always associated with behavioural arrest. These differences between our observed epileptiform discharges and the usual presentation of atypical absence seizures in mice may suggest that the discharge events we observed in the Mecp2<sup>+/</sup> mice are only typical absence seizures. The hippocampal correlation we found may just suggest that the hippocampus modulates discharge incidence rather than its circuitries recruited. There is evidence for this being the case, the hippocampus of MeCP2-null mice has been found to be prone to hyperexcitability and entrainment as well as displaying altered inhibitory rhythms (Zhang et al., 2008). As the hippocampus is known to project to the cortex, this altered hippocampal network in MeCP2-deficient mice may facilitate cortical entrainment and therefore SWD propagation in thalamo-cortical projections.

As absence seizures are known to originate in thalamo-cortical networks, the lack of a correlation between cortical MeCP2 levels and discharge incidence is unexpected. One possible explanation for this lack of correlation is that the decreased amount of mobility expressed by lower cortical MeCP2 levels obscures the cortical correlation with discharge incidence. As reported in this study, epileptiform discharges occurred much
more often in $\text{Mecp2}^{-/+}$ mice during an active behavioural state as opposed to an inactive state. We also found that lower cortical MeCP2 protein levels had a relatively strong correlative coefficient with reduced locomotive and exploratory behaviour in the $\text{Mecp2}^{-/+}$ mice. This reduced mobility caused by lower cortical MeCP2 levels will translate into a decrease in epileptiform discharges due to less time spent in an active state. It is therefore possible that the reduced amount of discharges due to decreased active state duration may mask an increase in epileptiform discharges associated with lower cortical MeCP2 levels.

4.1.4. Regional MeCP2 Correlation Data Suggests Behavioural Performance is Dependent on Multiple Systems

The correlational analysis between regional MeCP2 protein expressions and severity of specific behavioural impairments revealed significant correlations but they were never extremely strong. The majority of significant correlations found had $r$-values in the range of 0.7 to 0.9, suggesting that other brain regions contribute and play a role in the manifestation of that specific behavioural deficit. This is readily seen by the fact that we observe multiple brain regions correlating with only one specific behaviour in the $\text{Mecp2}^{-/+}$ mice, such as cortical and cerebellar MeCP2 levels both correlating with the number of daily delta cycles.

Additionally from our correlational analysis, we found instances where behavioural severity has reached a maximum or minimum state but the correlating region’s MeCP2 level are still below wild-type levels. For example, we observe no epileptiform discharges in some mice despite their hippocampal MeCP2 levels never
reaching wild-type amounts. On the other end of the spectrum, we also observed mice that displayed no centre rearing behaviour yet still possessed partial MeCP2 expression. These observations may suggest that other brain regions and systems contribute to the performance of the mouse in this specific behaviour. They may also suggest that there is a tolerance to MeCP2 deficiency in these brain regions before exhibiting an impaired behavioural phenotype. Similarly, there may also be a limit or minimum amount of MeCP2 that a region must express to be able to display its correlated behaviours in any degree.
4.2. Future Directions

Although our study clearly demonstrates that there are behavioural patterning deficits in \textit{Mecp2}\textsuperscript{+/−} mice, it did not examine whether sleep rhythms are impaired in these mice. As mentioned previously, high delta power is an indicator of sleep and immobility in rodents (Franken et al., 1998), but our results demonstrate a lack of correlation between activity and delta power in \textit{Mecp2}\textsuperscript{+/−} mice. It would therefore be beneficial to examine the sleep patterns of \textit{Mecp2}\textsuperscript{+/−} mice to assess whether they are disrupted as well as to determine the correlative strength of sleep to high delta power EEG signals. This could be achieved by repeating our same experiment but with the additional collection of electromyography (EMG) signals from muscles and 24 hour video recording to thoroughly establish that the mouse is in an awake or sleep state. Additionally, it would also be beneficial to examine whether light entrainment is altered in \textit{Mecp2}\textsuperscript{+/−} mice. From our results it seems that \textit{Mecp2}\textsuperscript{+/−} mice still express a preference towards increased activity during the dark phase but it would be worthwhile to further characterize the effects of light on these animals. The standards for assessing this are to expose the animals to either constant light or constant darkness instead of the usual 12:12h light/dark cycle and measure phase shifts in the onset of activity and free-running periods of the mice.

Another future direction for this research is to determine whether the abnormal daily behavioural patterning in \textit{Mecp2}\textsuperscript{+/−} mice can be rescued through pharmacological intervention. Two drugs that would be worthwhile to test are melatonin and diazepam. The major neurotransmitter in the SCN is GABA (Moore and Speh, 1993), and it has shown that GABA\textsubscript{A} receptors connect the local synaptic circuitry and contribute to the
synchronization of SCN firing (Jiang et al., 1997; Liu and Reppert, 2000). Melatonin, the main product of the pineal gland, can modulate the function of GABA<sub>A</sub> receptors (Wan et al., 1999) and injecting exogenous melatonin has been shown to promote free-running activity entrainment in rodents (Slotten et al., 1999; Carpentieri et al., 2006). Additionally, diazepam, which has a direct GABAergic effect on the SCN as well as a direct effect on melatonin synthesis in the pineal gland (Wakabayashi et al., 1991; Djeridane and Touitou, 2003), has also been shown to favor rodent entrainment to external light (Carpentieri et al., 2006). It is therefore interesting to see whether administration of these compounds rescues the behavioural patterning deficits we observed in M<sup>cp2<sup>−/−</sup></sup> mice.

As alluded to before, it is unclear whether the MeCP2 expression levels are a direct result of skewed XCI patterns. Therefore, a future direction for this research is to examine, compare, and correlate MeCP2 expression levels with XCI patterning in a more comprehensive collection of brain regions. The previous study from Young and Zoghbi (2004) showed that XCI patterns are uniform between two different brain regions and that XCI always favours the expression of the wild-type M<sup>cp2</sup> allele in M<sup>cp2<sup>308</sup></sup> mice. These are in contrast to our results from this study which show there is no uniform expression of MeCP2 protein levels between brain regions and that MeCP2 protein expression can be well below 50% of wild-type levels in M<sup>cp2<sup>−/+</sup></sup> mice. Despite the use of different animals models, this disparity in findings warrants further investigation into the relationship between M<sup>cp2</sup> XCI patterns and actual protein expression. It would therefore be worthwhile to repeat our study but with the additional measurement of the percentage of cells that express the wild-type M<sup>cp2</sup> allele in various brain regions of
In addition to examining XCI patterns, it would also be beneficial to widen the number of brain regions assessed and used in the correlations. From our findings in these studies, it would also be of interest to measure MeCP2 protein levels in the suprachiasmatic nucleus, hypothalamus, thalamus, as well as the basal ganglia and correlate these region’s MeCP2 protein levels with daily delta power and activity rhythms, thermoregulatory patterns, epileptiform discharge incidence, and motor deficit severity respectively. Conversely, targeted knockdown of MeCP2 through the use of small interfering ribonucleic acids (siRNAs) could be undertaken to examine the effects of abolishing MeCP2 expression in these specific areas on behavioural outcomes. Targeted knockout mouse models of MeCP2 could also be generated to characterize the extent of phenotype displayed by mice lacking MeCP2 in these specific regions similar to those already performed looking at Hox-B1 targeted MeCP2 knockout mice (Ward et al., 2011), aminergic neuron-specific MeCP2 knockout mice (Samaco et al., 2009), and forebrain specific MeCP2 knockout mice (Gemelli et al., 2006). This is achieved by crossing a loxP-flanked Mecp2 mouse with a mouse expressing Cre-recombinase linked to a tissue-specific promoter, such as the mammalian Period 1 (mPer1) promoter which is dominantly expressed in the suprachiasmatic nucleus (Travnickova-Bendova et al., 2002). Conversely, mouse models with targeted re-activation of Mecp2 in these brain
regions could also be generated. This may be achieved through the crossing of a mouse with a MeCP2 gene containing a loxP-flanked stop codon cassette and a mouse expressing a Cre-recombinase linked to a tissue-specific promoter. In these offspring mice, the mice would lack MeCP2 ubiquitously except in the tissue containing specific promoter regions expressing Cre. Comparing complete MeCP2-deficient mice against mice with targeted MeCP2 re-activation, we would be able to provide some insight into the possible benefits or improvements that could be expected from clinical MeCP2 gene therapy to these areas.
References


