ALTERATIONS OF CORTICAL AND HIPPOCAMPAL NETWORK ACTIVITY IN MECP2-DEFICIENT MICE

by

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

Institute of Medical Science

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ABSTRACT

Intractable epilepsy remains one of the top issues affecting the quality of living in Rett children. While several MeCP2-deficient mouse models of Rett Syndrome have been established, minimal information exists on how the loss of MeCP2 affects brain network activity. To address this issue, in vivo recordings of the hippocampus and somatosensory cortex of MeCP2-deficient mice were taken during exploration, immobility, and sleep. The frequency of hippocampal theta oscillations was significantly attenuated in MeCP2-deficient mice during exploration. A subset of MeCP2-heterozygotes displayed spontaneous, cortical epileptiform-like discharges in the immobile-awake state. Similar epileptiform-like discharges were observed in one of the four Mecp2-null mice recorded. Aside from these EEG abnormalities, basal network activity was preserved. Further, convulsive seizures were not seen. Collectively, these findings indicate that a deficiency of MeCP2 in mice leads to only subtle alterations in brain wave activity, contrasting the severely abnormal EEG observed in Rett girls.
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<tbody>
<tr>
<td>5H1AA</td>
<td>5-hydroxy-indoleacetic acid</td>
</tr>
<tr>
<td>5HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>ACh</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain derived neurotrophic factor</td>
</tr>
<tr>
<td>Ca2+</td>
<td>calcium ion</td>
</tr>
<tr>
<td>CA1</td>
<td>corono ammonis area 1</td>
</tr>
<tr>
<td>CA3</td>
<td>corono ammonis area 3</td>
</tr>
<tr>
<td>CFTR</td>
<td>cystic fibrosis transmembrane conductance regulator</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPS</td>
<td>complex partial seizure</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP response element-binding</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>DG</td>
<td>dentate gyrus</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalography, electroencephalogram</td>
</tr>
<tr>
<td>EPSC</td>
<td>excitatory postsynaptic current</td>
</tr>
<tr>
<td>EPSP</td>
<td>excitatory postsynaptic potential</td>
</tr>
<tr>
<td>ES</td>
<td>embryonic stem</td>
</tr>
<tr>
<td>FFT</td>
<td>fast fourier transform</td>
</tr>
<tr>
<td>HFS</td>
<td>high frequency stimulation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>HPC</td>
<td>hippocampus</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz (seconds⁻¹)</td>
</tr>
<tr>
<td>IGF-1</td>
<td>insulin-like growth factor 1</td>
</tr>
<tr>
<td>IPSC</td>
<td>inhibitory postsynaptic current</td>
</tr>
<tr>
<td>IPSP</td>
<td>inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>K⁺</td>
<td>potassium ion</td>
</tr>
<tr>
<td>LIA</td>
<td>large amplitude irregular activity</td>
</tr>
<tr>
<td>LTD</td>
<td>long term potentiation</td>
</tr>
<tr>
<td>LTP</td>
<td>long term depression</td>
</tr>
<tr>
<td>mM</td>
<td>millimolar (milimoles per liter)</td>
</tr>
<tr>
<td>MBD</td>
<td>methyl binding domain</td>
</tr>
<tr>
<td>MeCP2</td>
<td>methyl CpG binding domain protein 2</td>
</tr>
<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>Na⁺</td>
<td>sodium ion</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NR2A</td>
<td>NMDA receptor 2A</td>
</tr>
<tr>
<td>NR2B</td>
<td>NMDA receptor 2B</td>
</tr>
<tr>
<td>REM</td>
<td>rapid eye movement (sleep)</td>
</tr>
<tr>
<td>RT</td>
<td>reticular thalamic</td>
</tr>
<tr>
<td>RTT</td>
<td>Rett Syndrome</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SPS</td>
<td>simple partial seizure</td>
</tr>
<tr>
<td>SRFP</td>
<td>spontaneous rhythmic field potentials</td>
</tr>
<tr>
<td>SWD</td>
<td>spike and wave discharge</td>
</tr>
<tr>
<td>SWS</td>
<td>slow wave sleep</td>
</tr>
<tr>
<td>TC</td>
<td>thalamocortical</td>
</tr>
<tr>
<td>TRD</td>
<td>transcriptional repressor domain</td>
</tr>
<tr>
<td>XCI</td>
<td>X- chromosome inactivation</td>
</tr>
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</table>
1. INTRODUCTION

1.1. Rett Syndrome

1.1.1. Stages of Rett Syndrome

Rett Syndrome (RTT) is a neurodevelopmental disorder affecting 1 in 10,000 to 1 in 20,000 girls (Laurvick et al, 2006). It was first recognized as a unified clinical condition by Dr. Andreas Rett in 1966, but did not receive international attention until 1983 with the publication of Hagberg et al (1983).

In 1954, the Austrian pediatrician Dr. Andreas Rett observed two girls in his waiting room displaying the same repetitive hand-washing motions. Looking at their clinical charts, he realized they shared similar clinical and developmental histories. Dr. Rett soon recognized that six other girls in his practice had similar behaviours, and reasoned that these girls must have the same disorder, which at that time was new to the medical field (Hunter, 2006). In fact, their distinctive behavioural patterns indicated to him that this new condition was not a simple mental retardation syndrome, but rather a complex condition that affected several facets of neural function. Unfortunately, during the 1960’s in Europe the medical community was hesitant to recognize “new” conditions in the absence of a metabolic abnormality. This led Dr. Rett to examine several physiological parameters in his affected girls, where he found elevated ammonia content in blood. In 1966, Dr. Rett
published his observations in a leading German medical journal, where he described the condition as one of cerebral atrophy and hyperammonia in girls, characterized by autistic behaviour, dementia, and apraxia of gait (Rett et al, 1966). The condition stayed mostly unrecognized in the English language literature until 1983, when Bengt Hagberg revisited Rett’s unusual mental retardation syndrome and suggested the condition bear his name (Hagberg et al, 1983). While most of the symptoms recognized by Dr. Rett are still criteria for diagnosis today, high ammonia levels and cerebral atrophy are no longer considered features of RTT.

RTT follows a stereotypic progression and can be divided into four stages: (1) pre-regression, (2) regression, (3) post-regression and (4) late motor regression (Hagberg and Witt-Engerstrom, 1986).

(1) Pre-regression

One of the cardinal features of Rett Syndrome is a seemingly normal initial period of development. Rett girls typically appear normal for the first 6-18 months of their life. These girls may achieve normal developmental milestones, and even begin to speak and walk (Hagberg and Witt-Engerstrom, 1986). However, subtle changes may occur including decelerated head growth, and loss of interest in play.

(2) Regression
This is followed by a stage of rapid regression in which Rett girls lose previously acquired skills, such as rudimentary language, ability to grasp items, and purposeful use of hands. Developmental deterioration is prominent and there is marked slowing of head circumference growth. Rett girls are small for their age and the brain is abnormally undersized. Dementia and autism are noticeable at this stage. Rett girls are able to visually follow objects and people but show no sustained interest. Breathing irregularities such as apnea and hyperventilation occur, and Rett stereotypies such as hand-wringing, clapping, and hand-washing motion manifest themselves. Rett girls are usually able to walk although mobility is clumsy and uncoordinated (Hagberg and Witt-Engerstrom, 1986). This stage lasts until approximately 4 years of age, followed by a pseudo-stationary stage in which the progression of the disease appears to stabilize.

(3) Post-regression

The post-regression phase refers to a stage where the disabilities of Rett syndrome remain fairly constant. This phase typically lasts many years, and can be maintained until Rett girls reach their 20’s. During the post-regression phase, autism is less of a problem, and dementia improves. Emotional disturbances may be present, and Rett girls show unusual anxiety when confronted with a novel situation, or even without provocation (Hagberg and Witt-Engerstrom, 1986). Epilepsy is considered a later symptom of RTT and is prominent in this stage, occurring in approximately 70% of girls (Laurvick et al, 2006). Gait apraxia is
also highly common (Hagberg and Witt-Engerstrom, 1986). This stage is followed by late motor regression in which motor dysfunction is the primary pathology.

(4) Late motor regression

The pseudo-stationary post-regression phase is typically followed by stage in which remaining motor ability deteriorates. At this stage, mobility is greatly decreased and Parkinson-like behaviours develop. Most Rett girls are wheelchair bound, although modern therapy has greatly improved ambulation. Motor problems typically include scoliosis, a lateral curvature of the spine, spasticity, and hypertonicity of the lower limbs. Extremities are cold, red and clammy, suggesting autonomic misregulation, and feet become abnormally small (Hagberg 1983; Hagberg 1986).

Autonomic misregulation appears to be a crucial feature of RTT, causing breathing irregularities, heart rate variability, small cold feet, constipation, and difficulty swallowing (Julu, 2001). However, the neural systems responsible for autonomic misregulation remain unknown.

1.1.2. Neuropathology of Rett Syndrome

It is now known that the decreased head growth associated with RTT is not due to neurodegeneration, but rather to decreased neuronal growth. Rett neurons of the
hippocampus, thalamus, and cortex show reduced neuronal size, decreased dendritic territories, and increased neuronal packing density (Armstrong, 2005).

Neurotransmitter levels may be abnormal in the Rett brain, although studies on animal models are necessary for a controlled assessment of the neurochemistry of RTT (Armstrong, 2005). Studies on Rett girls found consistently low levels of acetylcholine in the brain. Decreased cholinergic markers were found in the basal forebrain, basal ganglia and midbrain tegmentum (Wenk, 1997). Also, cerebral blood flow is greatly reduced in Rett girls, particularly in the frontal lobe (Kaufmann, 2001), which is consistent with decreased cholinergic tone. Studies in Alzheimer’s patients have shown that selective neurodegeneration of cholinergic neurons underlies dementia and that treatment with acetylcholinesterase inhibitors, drugs that increase cholinergic transmission by blocking the breakdown of acetylcholine in the synapse, may improve memory function (Grutzlender and Morris, 2001). In Rett mice, dietary choline supplementation was found to improve motor function and increase brain volume (Ward et al, 2008). Choline supplementation improved grip strength in MeCP2-heterozygotes and motor coordination and hypoactivity in MeCP2-null mice (Nag and Berger-Sweeney, 2007). Choline supplementation may therefore be beneficial in the treatment of RTT, and may partially rescue neurological deficits such as dementia.
Deficiencies in monoamine neurotransmitters may produce specific behavioural alterations associated with Rett Syndrome (Samaco et al, 2009). Monoamine neurotransmitters are derived from aromatic amino acids and include serotonin (derived from tryptophan) and the catecholamines (dopamine, norepinephrine and epinephrine, all derived from tyrosine). Monoamine levels are significantly reduced in both Rett girls and mice (Zoghbi et al, 1989; Samaco et al, 2009; Ide et al, 2005). MECP2 is believed to regulate the expression of rate-limiting enzymes involved in the catalysis of catecholamine and serotonin neurotransmitters (Samaco et al, 2009).

Serotonin is primarily released from the raphe nuclei in the brainstem, and is associated with regulation of mood, sleep, appetite, respiration, etc. Serotonergic deficiency may account for mood disturbances, anxiety and aggression in RTT as alterations of serotonergic transmission are responsible for these behaviours in other conditions (Lucki 1998; Gordon and Hen, 2004; Popova 2008). A recent study found that levels of serotonin and its metabolite 5H1AA are significantly reduced in the hippocampus of MeCP2-null mice and pre-symptomatic MeCP2-heterozygotes (Isoda et al, 2009). The authors suggested that decreased serotonergic innervation to the hippocampus may account for anxiety and reduced exploration in Rett mice models. Samaco et al (2009) found that when MeCP2 was selectively removed from serotonergic neurons, resulting in decreased serotonergic synthesis, the resulting mutant mice displayed breathing abnormalities and were more
aggressive in the resident intruder test. Breathing disturbances may also arise in part from
a deficiency in serotonergic and noradrenergic modulation of the medullary respiratory
network (Viemari et al, 2005). *Mecp2*-null mice showed an initial decrease in medullary
levels of norepinephrine at 1 month of age when respiratory disturbances began, followed
by a secondary decrease in medullary serotonergic levels at 2 months of age, at which time
breathing disturbances had worsened considerably and mice were near death (Viemari et
al, 2005).

Respiratory arrhythmia accounts for approximately 26% of deaths in Rett Syndrome
(Kerr et al, 1997). Breathing patterns are normal during sleep but may be highly irregular
during wakefulness (Julu, 2001). Similarly, in *Mecp2*-deficient mice, breathing patterns are
normal under light anesthesia (Viemari et al, 2005). Norepinephrine is known to play a role
in the maturation and modulation of the medullary respiratory network (Viemari et al,
2004), and noradrenergic deficiency is believed to be primarily responsible for irregular,
depressed breathing in *Mecp2*-deficient mice (Viemari et al, 2005). Further, treatment of
*Mecp2*-null mice with desipramine, a tricyclic antidepressant most selective at blocking
norepinephrine synaptic reuptake, was found to improve breathing patterns and
significantly extend lifespan (Roux et al, 2007).

Studies have found less tyrosine hydroxylase, a marker of catecholaminergic
neurons (adrenergic, noradrenergic, or dopaminergic), in the substantia nigra pars
compacta and midbrain of Rett girls (Jellinger, 1986). Additionally, Rett girls have fewer neurons in the substantia nigra (Kitt and Wilcox, 1995). The substantia nigra pars compacta is a part of the basal ganglia, a group of nuclei best known for their function in motor control, and is principally composed of dopaminergic neurons. Neurodegeneration of the dopaminergic neurons of the substantia nigra pars compacta is associated with Parkinson’s disease, a movement disorder associated with motor rigidity, loss of voluntary movement and dystonia at the later stages (Jellinger, 1987). Decreased dopaminergic function in this structure may explain motor dysfunction and dystonia in Rett girls. In support, Rett girls respond to L-dopa, a catecholamine precursor which has become standard in the treatment of Parkinson’s. L-dopa has been found to alleviate motor rigidity in the late stages of RTT (Budden, 1997).

Excitatory synaptic activity may be abnormal in Rett girls. Seizures are common in the first decade of life and cortical hyperexcitability is observed. Glutamate levels were found to be elevated in Rett girls in CSF and MR studies (Hamberger et al, 1992; Pan et al, 1999). Additionally, the expression of glutamate receptors subtypes showed age-related trends (Johnston et al, 2005). Glutamate receptor levels were elevated in younger girls (<8 years) and were lower than controls in older girls (>8 years). NMDA receptors were especially elevated in the frontal cortex while AMPA receptors were most elevated in the basal ganglia in younger Rett girls. Elevated glutamate and glutamate receptor levels in
younger Rett girls may explain why these girls have a propensity to develop seizures in the first decade of their lives. GABA receptors showed a similar trend, and were elevated in younger girls and reduced in older girls, although to a lesser degree. High levels of excitatory and inhibitory transmitter receptors are typically expressed in very young children during synaptogenesis. Thus, elevated levels of glutamate and GABA receptors in Rett girls may reflect an immature brain state (Johnston et al, 2005). In Rett mice, the subunit composition of NMDA receptors was found to be altered (Asaka et al, 2006). Hippocampal slices from mature Rett mice showed an increased expression of NR2B subunits and reduced expression of NR2A subunits. NR2B is typically expressed in immature tissue; NR2A is typically expressed in mature tissue and is more effective at generating NMDA-receptor dependent LTP. In normal mice, there is a developmental shift from in the expression of NR2 subunits from NR2B to NR2A. Elevated NR2B subunit expression in the mature Rett mouse brain may reflect an immature brain state, supporting findings from Rett girls (Asaka et al, 2006).

### 1.1.3. Causal Gene

The genetic cause of RTT remained evasive until quite recently, largely because the inheritance pattern was chiefly sporadic. Then in 1999, studies on the few familial cases identified the X-linked gene encoding methyl-CpG binding domain protein 2 (MECP2) as the causal gene in RTT (Amir et al, 1999). Mutations in MECP2 are associated with over
80% of RTT cases. Further, mice lacking *MeCP2* display Rett-like symptoms, and this phenotype can be reversed with the reintroduction of *MeCP2* (Guy et al., 2007). MECP2 was traditionally thought to act as a transcriptional repressor, exerting its function by silencing the expression of methylated genes (Nan et al., 1998). According to this view, MECP2 localizes to the nucleus where it binds to methylated CpG dinucleotides, and recruits histone deacetylase complexes via interactor protein sin3A. As their name suggests, histone deacetylase complexes remove acetyl groups from histones, protein structures around which DNA wraps. This exposes a positive charge on histones, resulting in a tighter interaction with the negatively charged DNA. As a consequence, transcriptional activators are unable to bind the DNA, thus blocking gene transcription (Nan et al., 1998). The phenotype observed in Rett syndrome was therefore initially hypothesized to arise from the erroneous expression of a profile of genes that should have been “silenced” by MECP2. Unfortunately, gene expression profiling experiments have failed to reproducibly detect large numbers of mis-expressed genes in the RTT brain or in the brains of *MeCP2*-null mice, raising the possibility that MECP2 may serve functions other than transcriptional inhibition in the brain (Chadwick and Wade, 2007).

Support for this latter possibility has grown with several recent publications, in which MeCP2 was found to associate more predominantly with euchromatin than heterochromatin in cultured cells, and influence chromatin structure in a transcriptionally-favoring manner at some of its genomic binding sites (Yasui et al., 2007). Further, a recent
study directly suggests that MeCP2 may act as a transcriptional activator in at least some neurons. A microarray study of mRNA isolated from the hypothalamus of MeCP2-deficient and MeCP2-overexpressing mice found the majority of genes displaying altered expression were upregulated rather than down-regulated by MeCP2, and the MeCP2 protein was found to directly associate with the transcriptional activator CREB in these neurons (Chahrour et al, 2008). Thus, delineating precisely how MeCP2 functions in the brain remains a “hot topic”, and it remains an open question whether the pathophysiology of Rett syndrome arises solely from the loss of normal transcriptional regulation provided by MeCP2.

Recently studies suggest MeCP2 may function non-cell-autonomously. In MeCP2-heterozygotes, neurons harbouring a mutated MeCP2 allele may reduce the expression of MeCP2 in wild-type neurons (Braunschweig, 2004). Furthermore, in mice chimeric for MeCP2-null and wild-type cortices, layer 2/3 pyramidal cells were smaller and showed reduced dendritic arborization in both wild-type and MeCP2-deficient neurons (Kishi and Macklis, 2009).
Figure 1. Structure of MECP2 gene shown above. MECP2 contains four exons. The two MECP2 isoforms, with and without exon 2 are shown below. The short isoform is expressed ubiquitously, while the long isoform is expressed predominantly in the brain.
MECP2 contains four exons and encodes two isoforms, a longer 498 amino acid protein and shorter 486 amino acid form (see figure 1). In mice, a single 484 amino acid homolog is found. MECP2 contains two functional domains, a methyl-binding domain (MBD) and the transcription repression domain (TRD). The MBD is located on exons 3 and 4, and binds to methylated CpG dinucleotides. The TRD is found on exon 4 and recruits histone deacetylase complexes (http://genome.ucsc.edu/). The majority of point mutations in RTT occur in exons 3 and 4 of the MECP2 gene, with large deletions occurring most commonly in the C-terminal (Percy et al, 2007).

1.1.4. Factors Influencing Phenotypic Outcome

Classic Rett Syndrome is associated with stereotypic hand movements, loss of purposeful movement, loss of speech, apnea, inability to walk, mental retardation, abnormal EEG and often epilepsy (Kerr and Witt-Engerstrom, 2001). However, the extent of variability in the Rett phenotype observed is huge. Milder forms of RTT are now being recognized that would have previously escaped diagnosis. These include the “preserved speech” variant form of Rett syndrome, as well as a host of forms in which mental retardation is observed, but speech and ambulation are largely preserved (Scala et al, 2007). The variation observed in RTT is likely caused by the interaction of several
factors including genetic background, type of mutation present, X-chromosome inactivation (XCI) and environmental exposure.

1.1.4.1. Mutation Type

A variety of MECP2 mutations are known to occur in RTT, including point mutations, insertions and deletions. Point mutations, which involve a single amino acid substitution, account for ~60% of observed MECP2 mutations (Bebbington, 2008). Point mutations can be further divided into missense mutations, in which one amino acid is substituted for another, nonsense mutations, in which a stop codon is incorrectly inserted in place of an amino acid, and silent mutations that do not seem to change an amino acid sequence, but may affect mRNA stability. Bebbington et al (2008) examined the genotype-phenotype relationship in 276 Rett girls harbouring seven common point mutations, R106W, R133C, T158M, R168X, R255X, R270X, R294X or R306C, or C-terminal deletions. Overall, Rett girls harbouring R133C, R294X or R306C mutations, or C-terminal deletions, displayed the mildest phenotype. Rett girls in the R270X category had the highest overall severity score.

At one end of the spectrum, girls with the R133C or R294X mutations were most likely to show delayed (after 3 years of age) onset of the regression phase, along with delayed onset of hand stereotypies; all girls in these categories learned to walk. The R133C category showed the greatest preservation of language, with 18% retaining the use of single
words, and 21% able to use phrases. Feeding difficulties were least common in girls with the R133C or R306C mutations. At the other end of the spectrum, just over one third of girls with R255X and R270X mutations learned to walk. In the R168X group, less than a third of girls learned to walk. Severe feeding difficulties were present most often in these three groups. Other common features of RTT such as epilepsy prevalence, scoliosis, breathing problems, peripheral circulation problems, mood disturbances and sleep problems, did not show a significant genotype-phenotype correlation (Bebbington, 2008).

1.1.4.2. Genetic Background

Genetic background may act as a disease modifier in RTT. Genetic polymorphisms in the gene encoding brain derived neurotrophic factor (BDNF) were found to correlate with the severity of RTT (Zeev et al, 2009). Presumably, other genetic polymorphisms, unknown as of yet, may also modify the course or severity of Rett Syndrome.

BDNF is a neurotrophic factor which promotes neurogenesis, neuronal survival, and plasticity in the brain. During neuronal activity, Ca2+ influx through voltage-sensitive Ca2+ channels activates transcriptional factors which in turn activate the BDNF promoter, resulting in transcriptional upregulation of BDNF (Tabuchi et al, 2000). MECP2 is believed to act as a negative transcriptional regulator of BDNF, binding to and repressing the BDNF promoter in the absence of membrane depolarization (Chen et al, 2003). Mutations to MECP2 which yield a nonfunctional protein should in theory elevate levels of BDNF.
However, BDNF is reduced to 70% of wild-type levels in whole brain preparations from *MeCP2*-deficient mice (Chang et al, 2006). This apparent contradiction may be explained by reduced synaptic activity in the Rett brain, which in turn produces less transcriptional upregulation of BDNF. Studies have shown that cortical activity is reduced in the Rett mouse brain (Dani et al, 2005).

Given the postulated role of BDNF misregulation in the etiology of Rett syndrome, it is worth noting that there is a naturally occurring polymorphism of the BDNF gene that affects the efficiency of BDNF processing. This polymorphism occurs at amino acid 66, and changes the normal valine moiety into a methionine. The V66M polymorphism occurs in the pro-BDNF sequence, an amino acid sequence responsible for intracellular targeting of BDNF. BDNF proteins possessing the V66M mutation were found to aggregate in the cell body, whereas wild-type proteins co-localized with synaptophysin, a marker of the synapse, in secretory vesicles (Egan et al, 2003). The V66M polymorphism thus impairs activity-dependent secretion of BDNF by interfering with intracellular sorting of the protein. The V66M mutation has been shown to lead to increased susceptibility to various neuropsychiatric conditions, including Alzheimer’s, Parkinson’s, bipolar, depression and obsessive compulsive disorder (Chen et al, 2004). This allele is associated with an 11% decrease in hippocampal brain volume, and poorer performance on hippocampal dependent memory tasks (Bueller et al, 2006). In Rett girls, the V66M polymorphism is
associated with earlier onset of seizures and a slightly more severe phenotype, particularly in girls harbouring the R168X MECP2 mutation (Zeev et al, 2009). However, it should be noted that another group found exactly the opposite and concluded that the V66M polymorphism was protective against early seizure onset (Nectoux et al, 2008).

1.1.4.3. X-Chromosome Inactivation

Only one functional X chromosome is needed in every cell of the human body. Therefore, in the female fetus a process called X-chromosome inactivation occurs, whereby an X-chromosome is randomly silenced in each cell of the developing embryo, although certain genes on the silenced chromosome may still be expressed (Lyon, 2008). Skewing of XCI may occur and in Rett can produce a pattern favouring the expression of the X-chromosome containing the normal MECP2 gene, or mutated MECP2 gene. Asymptomatic carriers of RTT were found to have highly skewed inactivation patterns of XCI favouring the normal MECP2 allele (Zoghbi et al, 1999). Sisters with identical MECP2 mutations were found to have extremely discordant phenotypes as a result of XCI skewing. One sister displayed classic Rett Syndrome while the other was a “highly functioning” preserved speech variant as a result of favourable XCI skewing (Scala et al, 2007). XCI patterns may interact with mutation type, so that Rett patients with severe early truncating mutation present a milder phenotype than would be expected. The process of XCI occurs in Mecp2-deficient female mice models and the degree of skewing has been shown to correlate with
phenotypic expression (Young and Zogbhi, 2004). In MeCP2-heterozygotes, XCI patterns generally favour the wild-type allele which may in part explain the mild phenotype observed (Young and Zogbhi, 2004).

1.1.4.4. Environment Enrichment

Environmental factors may also play a role in the phenotype of RTT. Music therapy has long been established as an effective method of relieving anxiety in Rett girls (Wesecky, 1986). Furthermore, music may motivate learning and environmental interaction in Rett girls (Elefant and Wigram, 2005; Lotan and Shapiro, 2005). In one study, seven girls with classic Rett Syndrome were taught to associate songs with symbol cards. Rett girls were then asked to select a symbol card and choose which song was to be played, either by eye gaze, nose pointing, or by hand. All seven girls were able to learn decision making in this paradigm (Elefant and Wigram, 2005). Lotan and Shapiro (2005) have suggested that young Rett girls may benefit from an enriched multi-sensory (Snoezelen) environment with a therapist. Such an environment is believed to promote relaxation, reduce anxiety, and allow exploration (Lotan and Shapiro, 2005).

In support, studies on Rett mice have found environmental enrichment to be beneficial (Nag et al, 2009; Kondo et al, 2008). MeCP2-deficient mice and wild-type mice were housed either in standard caging or in larger home cages with a variety of objects of differing texture, size and shape. In MeCP2-heterozygotes, environmental enrichment was
associated with improved performance on the accelerating rotarod test, a test of motor coordination, and with increased cerebellar levels of the neurotrophin BDNF (Kondo et al, 2008). In *MeCP2*-null mice, environmental enrichment was associated with subtle locomotory improvement (Nag et al, 2009). While environmental enrichment did not alter whole brain volume, ventricular volume was significantly reduced in *MeCP2*-deficient, particularly in the *MeCP2*-null group, suggesting that brain matter had increased (Nag et al, 2009).

### 1.2. Rett Mice Models

#### 1.2.1. *MeCP2*-null mice

With the discovery of the causal gene of RTT, several *MeCP2*-deficient mice models of Rett Syndrome have been developed. In North America and Scotland, three distinct Rett mice models have been established (Guy et al, 2001; Chen et al, 2001, Shahbazian et al, 2002). All of these mutant mice were created using the technique of Cre-loxP recombination.

Cre-loxP recombination is a valuable tool for creating general or conditional knockouts (Nagy, 2000). In this technique, a target genetic sequence is flanked with 34 base pair loxP sites. Recombination of the loxP sites occurs in the presence of a Cre recombinase enzyme, most commonly resulting in deletion of the targeted genetic sequence. The Cre
recombinase gene and loxP sites are often separately introduced to produce the desired knockout mutant. Typically, a targeting vector designed to insert loxP sites around a target gene (or portion of the gene) is transfected into embryonic stem (ES) cells. Correctly targeted ES cells are then injected into developing blastocysts to create chimeric mice. With selective breeding, a mouse line heterozygous or homozygous for the floxed gene is established. These mice are then crossed with a Cre recombinase-expressing line to produce the desired knockout phenotype (Nagy, 2000).

In Dr. Bird’s laboratory, mice with deletions of exons 3 and 4 of MeCP2 (which comprises nearly the entire protein) were created (Guy et al, 2001). Dr. Jaenisch’s lab produced a MeCP2-deficient mice model in which exon 3, which contains most of the MBD domain, was deleted (Chen et al, 2001). Both models produce MeCP2-null mice and MeCP2-heterozygotes, and result in similar phenotypes. Male MeCP2-null mice develop tremors, uncoordinated gait and have difficulties in motor tasks between 3-8 weeks, and later develop hindlimb clasping (an impairment in a standard reflex test in which mice suspended in the air by their tail will clasp their hindlegs together instead of spreading them out as normal mice would in preparation for landing) and irregular breathing (Guy, 2001; Chen 2001). A nearly identical phenotype was observed when MeCP2 was deleted in forebrain neurons only (Chen et al, 2001).
These mice models display a similar morphology to Rett patients with reduced brain and neuronal size. Total brain weight was reduced 9 to 13% compared to wild-type controls. Cortical brain volume was decreased between 7 to 11% and hippocampal volume was decreased 8% (Belichenko et al, 2008). By comparison, an autopsy study of Rett girls found brain weight to be reduced 12 to 34% relative to age-matched controls (Jellinger et al, 1988). Weight is abnormal in $Mecp2$-null mice and varies according to genetic background. $Mecp2$-null mice maintained on a C57Bl/6 background are undersized compared to wild-type littermates at the age of 4 weeks, whereas C57Bl/6 mice crossed with x129 strains were normal at this age and became overweight around 8 weeks of age. With both backgrounds, rapid weight loss occurred prior to death between 60 to 90 days (Guy et al, 2001; Chen et al, 2001). It should be noted that factors beyond the scope of this text have resulted in a modified progression of Rett symptoms in $Mecp2$-null mice, so that later generations have a slightly increased lifespan.

1.2.2. $Mecp2$-heterozygotes

The phenotype of $Mecp2$-heterzygous mice is much less severe and far more variable, likely due to XCI. Heterozygous mice generally develop Rett-like symptoms between 3 to 9 months which include hypoactivity, hindlimb clasping and weight
abnormalities. Also, these mice are fertile unlike their male counterparts (Guy et al, 2001; Chen et al, 2001).

1.2.3. *Mecp2*<sup>308/Y</sup>

Dr. Zoghbi’s lab created a Rett mouse model with a truncating mutation of MeCP2 (Shahbazian et al, 2002). The *Mecp2<sup>308/Y</sup>* mouse produces a 308 amino acid MeCP2 protein, in which the MBD, TRD and nuclear localization signal are retained. These mice are a better genetic model of RTT, producing a mutated MeCP2 protein which retains some functionality. *Mecp2<sup>308/Y</sup>* mice show a similar phenotype to their null counterparts, albeit delayed. Tremor and kyphosis (hunched back) develop around 5 months, and motor function assessed by a modified rotarod test, wooden dowel test, and wire grip test, was impaired. Clinical seizures were also reported in *Mecp2<sup>308/Y</sup>* mice. Most *Mecp2<sup>308/Y</sup>* mice survived past 1 year.

1.2.4. Findings from Rett Mice

*Mecp*2-deficient models are reminiscent of RTT in that autonomic misregulation occurs (tremor, breathing irregularities, weight abnormalities), smaller brain and neuronal size is observed, posture and motor coordination are abnormal, and seizures may be present (Guy et al, 2001; Chen et al, 2001; Shahbazian et al, 2002). Additionally, Rett mice
show a similar developmental progression to Rett girls, appearing normal at birth, achieving motor and developmental milestones early on, and showing a delayed onset of symptoms. *Mecp2*-deficient mice show similar neurochemistry to Rett girls; cholinergic markers are low in the brains of Rett girls and Rett mice show phenotypic improvement in response to dietary cholinergic supplementation (Wenk et al, 1997; Nag and Berger-Sweeney, 2007). Also, studies on Rett mice models are consistent with the view that RTT is mainly a synaptic disorder. Synaptic plasticity is impaired in Rett mice models, dendritic spines, the principal site of excitatory neurotransmission, are greatly reduced, and there appears to be an overall imbalance between excitation and inhibition (Armstrong et al, 2005, Dani et al, 2005).

1.3. Electrophysiology of Rett Syndrome

1.3.1. EEG in humans

An electroencephalogram (EEG) displays the summed, weighted field potentials from excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) on neuronal circuits close to a recording electrode (Holmes and Khazipov, 2007). There is an inverse relationship between a field potential’s contribution to the EEG and its distance from the recording site. Field potentials farther from the recording electrode tend
to have less of an impact on the EEG signal. The basics EEG principles can be understood by considering the electrical activity generated by a single neuron. Electrical activity in the brain is guided by source/sink coupling. Depolarization at a local portion of the neuronal membrane creates a local inward current or "sink". Current must complete a loop and flow from a sink to a source at another portion of the neuron as shown in figure 2. During an EPSP, recording electrodes positioned near the sink will be display an upward deflection on the EEG, while electrodes positioned close to the source will record a downward deflection during the EPSP. Whether an upward deflection or downward deflection is present in the EEG therefore depends on the positioning of the electrode. In neurons, EPSPs generally occur in the dendritic territories while IPSPs are directed to the soma (Holmes and Khazipov, 2007). The cerebral cortex has a laminar organization with the cell bodies of pyramidal neurons located in a particular layer at a particular depth, and their dendritic territories in another layer. The layered structure of the cortex allows larger amplitude signals to be produced when neighbouring neurons receive similar inputs and display similar firing patterns.
Figure 2. Principles of electroencephalography (EEG). The polarity of the EEG signal recorded depends on the position of the recording electrode. Current must complete a loop, flowing from sink to source. In neurons, EPSPs generally occur in the dendritic territories. During an EPSP, depolarization creates a “sink” or inward current at that portion of the membrane. Extracellular electrodes will display a downward deflection. Recording electrodes positioned near the “source” will show the opposite polarity.
Oscillatory patterns in the EEG reflect the synchronous activity of hundreds of neurons. However, the principles behind most EEG oscillations can be understood by considering the interactions of two types of neurons, an inhibitory (interneuron) and an excitatory neuron (principle cell). Four basic patterns of connectivity emerge (1) feedback inhibition (2) feedforward inhibition (3) mutual inhibition and (4) mutual excitation (Kandel et al, 2001). (1) In feedback inhibition, an excitatory neuron excites an inhibitory neuron, which in turn inhibits the firing of the principal excitatory neuron. (2) Feedforward inhibition involves a disynaptic delay in inhibition. An excitatory input excites both a principal cell, and inhibitory interneuron. The inhibitory interneuron then inhibits the excitatory neuron. The disynaptic delay prevents temporal summation in the principal cell, a process whereby membrane potential depolarizations caused by consecutive EPSPs summate over time until firing threshold is reached. (3) Mutual inhibition is very common in the central nervous system (CNS). It occurs when two inhibitory neurons hyperpolarize each other. Hyperpolarization in one neuron may deinactivate voltage-dependent channels, allowing burst firing which in turn deinactivates voltage-dependent channels in the other neuron, creating an oscillatory firing pattern. (4) The last pattern, mutual excitation, is uncommon in the normal brain but may be an important component of seizure activity. It occurs when one excitatory cell excites another, which in turn excites the first cell. Neuronal synchrony may be physiological or pathological. Excessive synchrony produces epileptiform activity such as spikes and sharp waves (Kandel et al, 2000).
The typical scalp EEG in humans oscillates at multiple frequencies. Perhaps the most prominent oscillations in humans are alpha waves (8-13 Hz) which typically occur over the occipital region when a person is in an awake, relaxed state. A more irregular, low-amplitude rhythm known as beta waves (13-30 Hz) is seen in awake, alert adults as well as during rapid-eye movement (REM) sleep. During slow wave sleep (SWS) delta waves (0.5-4Hz) are recorded across the cortex. Theta waves (4-7Hz) also occur during sleep and are believed to be involved with learning and memory (Kandel et al. 2000).

1.3.2. Seizures

A seizure is defined by the International League Against Epilepsy (ILAE) as the transient occurrence of symptoms or signs due to abnormal excessive or synchronous neuronal activity of the brain (http://www.ilae-epilepsy.org/). Seizures are generally self-limiting events except in the case of status epileptus. Seizures can have many triggers; however, for a person to be diagnosed with epilepsy they must have had at least one seizure and have a predisposition to generate seizures due to alterations in the brain (Fischer et al, 2005). The EEG during a seizure episode is termed “ictal”, and the EEG recorded in the period proceeding seizure activity is termed “post-ictal”. The EEG between seizure events is termed “inter-ictal” and may show epileptiform abnormalities such as single spikes. Inter-ictal sub-seizures often provide clues on the site of seizure origination (Fischer et al, 2005). Scalp recordings pick up globalized patterns near the surface of the
brain. Consequently, the EEG correlates of seizure activity are often not detected by scalp recordings, especially if seizures are localized to deep brain structures or confined to a small region of the cortex.

Seizures can be subdivided into two broad categories based on the extent of cortical involvement (http://www.ilae-epilepsy.org/). (1) Partial seizures are restricted to one hemisphere. (2) In contrast, generalized seizures may originate at one foci but rapidly spread to both hemispheres and are bilateral. (1) Partial seizures are broadly divided into simple partial seizures (SPS) and complex partial seizures (CPS). During a SPS, consciousness is intact and the person feels rhythmical twitching, or unusual tastes or sensations. CPS affects consciousness and usually involves the temporal lobe. These seizures may or may not be preceded by “aura”, and involve automatisms. (2) The two most common types of generalized seizures in the general population are tonic-clonic and absence epilepsy, formerly known as grand mal and petit mal respectively. In absence epilepsy, brief interruptions of consciousness are accompanied by a 3/s spike and wave complexes on the EEG (Wolf, 1985). These seizures are very brief, and are accompanied by a normal post-ictal phase. Tonic-clonic seizures occur in two phases. The tonic phase is characterized by muscle stiffening. Consciousness is lost and the person usually falls. In the proceeding clonic phase, body extremities rhythmically jerk and twitch. Both tonic seizures and clonic seizures can also occur in isolation. Myoclonic seizures occur without loss of consciousness and cause repetitive jerking of muscles (Wolf, 1985).
1.3.3. EEG Abnormalities and Epilepsy in Rett Syndrome

Seizures are highly common in RTT, occurring in about 70% of Rett girls (Jian et al, 2007). For the most part, seizures are manageable with common anti-epileptic drugs such as carbamazepine, lamotrigine and valproate. The prevalence of drug-resistant epilepsy was found to be 16% among Rett girls, lower than the general epileptic population (20 to 40%) (Jian et al, 2007). However, our current understanding of seizures in RTT is very limited. Nearly all seizures types have been reported; one study of 50 Rett girls with a history of epilepsy reported all seizure types, except absence and clonic seizures (Steffenburg et al, 2001). Inter-ictal spikes in Rett girls are often multifocal, suggesting there is no specific site of seizure origination. Also, multiple seizure types commonly occur in Rett girls.

A video-EEG study of 55 Rett girls demonstrated that seizures may be underrecognized or overestimated in Rett Syndrome (Glaze, 1998). Behavioural manifestations of clinical EEG seizures were not recognized in 15% of cases (Glaze, 1998). On the other hand, behaviours such as twitching, jerking, head turning, trembling, laughing, breath-holding, and hyperventilation, described as “typical” seizures by parents, were not associated with abnormal EEG discharges in 42% of cases. Another study found that vacant spells, traditionally associated with absence epilepsy, were nonepileptic in 14% of classic Rett Syndrome cases (Cooper et al, 1998). Many of these “nonseizure” behaviours
are thought to be manifestations of autonomic nervous dysfunction. One explanation is that recurrent seizures and interictal epileptiform discharges in limbic structures alter autonomic nervous system function via projections to the hypothalamus, resulting in these behaviours (Devinsky et al, 1994).

The Rett brain is hyperexcitable and Rett girls show “giant” amplitude somatosensory evoked potentials, although this was not seen with older patients. There is a characteristic progression of EEG abnormalities in the Rett brain, often paralleling the clinical progression of RTT (Glaze, 2005). For this reason, EEG abnormalities will be considered in four stages.

(1) Pre-regression

The EEG in the pre-regression stage is usually not studied as Rett Syndrome is typically not diagnosed until the age of 6-18 months when symptoms first appear.

(2) Regression

During the regression phase, there is a slowing of background activity during wakefulness and loss of the occipital dominant rhythm (also known as alpha waves). There is also progressive loss of non-REM sleep characteristics including sleep spindles and vertex transients. During slow wave sleep, focal spike or sharp wave discharges may occur independently or synchronously in both hemispheres (Glaze, 2005).
(3) Post-regression

In the post-regression stage, there is a marked slowing of background activity. Multifocal spike and or sharp wave discharges are present in the awake state, and a generalized slow spike-wave pattern emerges during slow wave sleep towards the end of this stage (Glaze, 2005). Hagberg et al (1983) also reported a decrease in EEG voltage during wakefulness in this stage (3-8 years) which in some patients was interrupted by bursts of high amplitude slow waves. Seizures typically appear in this stage, starting at 4 years of age.

(4) Late motor regression

EEG abnormalities and seizures may improve in the final stage of Rett Syndrome. Some Rett girls even show normal EEG patterns and have no seizures in this stage. However, in other girls EEG abnormalities are present. These include multi-focal spikes or sharp wave discharges or generalized slow spike wave activity, and continuous generalized slow spike wave activity during slow wave sleep. Another characteristic EEG pattern prominent in older Rett girls is a fronto-central slow activity (3-6Hz) (Glaze, 2005).

1.4. Electrophysiology of Rett Mice

1.4.1. EEG in mice

1.4.1.1. Hippocampus
Figure 3. (Modified from O’Keefe, 2006). Structure of the hippocampus. The hippocampus includes the entorhinal cortex (EC), dentate gyrus (DG), CA1, CA2, CA3 and subiculum subregions. Various pathways connect each these regions. Information flows from the entorhinal cortex to the dentate gyrus via the perforant pathway. Mossy fibres connect the dentate gyrus to the CA3 region. The CA3 projects to the CA1 via the schaffer collaterals, which in turn projects to the entorhinal cortex to complete the informational loop. It should be noted that several parallel loops are also present but this describes the bulk of information flow in the hippocampus.
The hippocampus is three or four layered structure (O'Keefe, 2006). In the CA1 region, the soma of principal (excitatory) cells is located in the pyramidal cell layer. Directly above is the stratum oriens layer, which contains the basal dendrites of pyramidal cells. Below the pyramidal cell layer is the stratum radiatum, which contains dendrites of pyramidal cells, various interneurons, and CA3 to CA1 projections. The deepest layer of the CA1 is the stratum lacunosum-moleculare which contains various inhibitory interneurons, as well as projections from the entorhinal cortex (O'Keefe, 2006).

The hippocampus is one of the most studied regions of the brain, and its circuitry is well established. There is a unidirectional flow of information (see figure 3). Input from the cortex reaches the hippocampus via the entorhinal cortex. The entorhinal cortex sends reciprocal connections back to the cortex and serves as the main output structure of the hippocampus. The entorhinal cortex primarily projects to the dentate gyrus via the perforant pathway, although it also projects to all other regions of the hippocampus (CA3, CA1 and subiculum). The dentate gyrus in turn projects to the CA3 region via mossy fibers, and the CA3 projects to the CA1 via the schaffer collateral pathway. The CA1 then projects to the subiculum which projects back to the entorhinal cortex, completing the loop. The CA1 also projects to the entorhinal cortex, creating a parallel loop (O'Keefe, 2006).

The hippocampus and other limbic structures are known to have the lowest seizure threshold based on kindling studies (Gilbert, 2001). The CA3 region of the hippocampus is
particularly vulnerable to seizure generation; recurrent axon collaterals between CA3 pyramidal neurons can produce a pattern of mutual excitation (O’Keefe, 2006). The CA3 region is unique in that bursting of CA3 pyramidal cells occurs in the absence of synaptic input. When the entire network of CA3 pyramidal neurons becomes synchronously active, as is the case when synaptic inhibition is blocked, a large amplitude depolarization known as a paroxysmal depolarization shift (PDS), results (O’Keefe, 2006). PDS is a hallmark of seizures.

In humans the hippocampus is necessary for acquisition of declarative memories, memories of facts and events, while in animal models its best known function is spatial learning. Three behavioural-dependent EEG states occur in the rodent hippocampal brain, although only two have been well characterized (O’Keefe, 2006). (1) The hippocampal theta rhythm is present in the hippocampus and entorhinal cortex during exploration and REM sleep. (2) Large amplitude irregular activity (LIA) occurs during consummatory behaviours, immobility, and slow wave sleep. (3) Small amplitude irregular activity occurs during transitions between behavioural states and is poorly understood.

(1) Theta

Theta is clinically defined as a relatively high voltage, sinusoidal waveform between 6-12Hz (Buzsaki, 2005). It should be noted that the theta range in rodents differs from that of humans (4-7Hz). The power spectrum of exploratory theta is characterized by a sharp
peak between 7 to 10 Hz, and a smaller second peak around 16Hz corresponding to the second harmonic (O’Keefe, 2006). When recording electrodes are positioned in the hippocampal pyramidal cell layer, the resulting theta waveform reflects the summed activity of IPSPs and EPSPs on the soma and dendrites of principle cells.

At the cellular level, the generation of the hippocampal theta rhythm relies on the synchronous, phasic firing of “theta” cells. Theta cells have a firing rate between 10Hz and 100Hz (during exploratory behaviours associated with theta). The bursting of these cells shows a consistent correlation with a particular phase of the theta wave. Theta cells in the CA1 and dentate gyrus (DG) regions are locked into synchrony by an inhibitory interneuron network consisting of GABAergic interneurons (O’Keefe, 2006).

Spatial mapping in the hippocampus relies on the interaction between theta cells and a second type of pyramidal cell found in the hippocampus, complex spike cells. Complex spike cells, also known as place cells, are “silent” or have a very low, irregular baseline firing rate. However, when an animal enters a particular location in a familiar environment, called a place field, the cell fires in rhythmic bursts at a frequency slightly higher than that of the EEG theta rhythm, so that its spikes correspond to progressively earlier points on the theta wave. The phase of these spikes relative to the EEG theta clock progresses as the rodent traverses the place field, a phenomena called phase precession. Phase precession encodes spatial location within a place field (O’Keefe and Reece, 1993).
Theta has two components based on pharmacology, an atropine-sensitive component and an atropine-resistant component. The atropine-sensitive component is blocked by the administration of muscarinic ACh receptor blocker atropine, while the atropine-resistant component is abolished by urethane anesthesia and similarly by NMDA receptor blocker ketamine. We now know that these components rely on the cholinergic input from the medial septum, and glutamatergic input from the entorhinal cortex respectively (Buzsaki, 2006). The medial septum is thought to act as a pacemaker and sends cholinergic projections, onto excitatory neurons such as the granule cells of the dentate gyrus, and GABAergic projections onto inhibitory interneurons of the hippocampus (O'Keefe, 2006).

Changes to the amplitude or frequency of hippocampal theta adversely affect the functioning of the hippocampus. When the hippocampal theta rhythm is eliminated completely through lesions to the medial septum, rodents are unable to perform previously learned spatial navigation tasks, suggesting that the hippocampal theta rhythm is necessary for spatial memory (Winson, 1978). The frequency and amplitude of the EEG theta wave is regulated by excitatory inputs from the entorhinal cortex (glutamatergic) and medial septum (cholinergic), and inhibitory (GABAergic) input from septohippocampal neurons.

*Cholinergic modulation of theta*
Atropine decreases the amplitude of hippocampal theta; its effect on theta frequency is modest (Lee et al, 1994). Conversely, drugs which increase cholinergic transmission enhance theta oscillations. There are several explanations as to how cholinergic transmission modulates theta (Buzsaki, 2002). One explanation is that medial septal cholinergic projections depolarize principal cells and interneurons of the hippocampus, resulting in theta oscillations. Another explanation is that tonic cholinergic excitation of inhibitory interneurons coupled with phasic septal GABAergic inhibition produces rhythmic discharges of GABAergic interneurons, which impose rhythmic IPSPs on principal cells. A third explanation is that the cholinergic component of theta relies on the recurrent CA3 collateral system which acts as a rhythm generator. Intrinsic CA3 oscillations are conveyed to other regions of the hippocampus through intrahippocampal associational projections to the CA1 stratum radiatum layer and dentate gyrus molecular layer (Buzsaki, 2002).

**GABAergic modulation of theta**

Drugs that relieve anxiety by potentiating GABA<sub>A</sub> receptor mediated inhibition, such as benzodiazepines, lower the frequency of theta oscillations and have an anti-cognitive effect (McNaughton et al, 2007). A variety of GABAergic interneurons are involved in theta modulation. Some interneuron types provide phasic, perisomatic inhibition to principal cells via GABA<sub>A</sub> mediated IPSPs. Other interneuron types terminate on innervation zones
of entorhinal afferents on distal dendrites of pyramidal and granule cells. GABAergic interneurons phase-lock theta cells in the hippocampus through IPSPs (Buzsaki, 2002).

**Glutamatergic modulation of theta**

Drugs that block glutamatergic receptors, specifically NMDA receptors decrease both the frequency and amplitude of theta oscillations (Buzsaki, 2002). Excitatory glutamatergic input from the entorhinal cortex is believed to drive the atropine-resistant component of theta. Entorhinal cortex cells have their own intrinsic oscillations; layer II stellate cells exhibit voltage-dependent oscillations in the theta frequency range. In the CA1, theta is driven in part by distal dendritic excitation of pyramidal cells via the perforant pathway. This excitation is mediated by both AMPA and NMDA receptors (Leung and Shen, 2004). CA3 projections onto the proximal CA1 dendrites also utilize excitatory glutamate transmitters.

**(2) Large Amplitude Irregular Activity (LIA)**

The other EEG pattern commonly seen in the hippocampus, LIA, is characterized by a broad power spectrum in the lower frequency range (between 1-5Hz) (O’Keefe, 2006). Sharp waves occur during all LIA activities, although they occur more frequently during slow wave sleep. Sharp waves are bursts of hypersynchronous activity, between 50 to 100 ms in duration, which resemble the inter-ictal spikes seen in epileptic cortices. They are believed to be involved in memory consolidation (Buzsaki, 1986). Sharp waves reach their
maximum amplitude in the stratum radiatum layer of the hippocampus, and reverse polarity in the pyramidal cell layer. Sharp waves are believed to originate in the CA3, and spread to the CA1 via the schaffer-collateral pathway. Synchronous, fast oscillations (120-200Hz) termed “ripples” occur shortly after the onset of sharp waves. Ripples are more prominent in the CA1 subregion, and have their maximum amplitude in the pyramidal cell layer. Ripples reflect the membrane potential oscillations of CA1 pyramidal cells caused by IPSPs from inhibitory interneurons that are synchronously activated by CA3 pyramidal cells shortly after sharp wave onset, and then project to the soma of CA1 pyramidal cells. The generation of ripples also relies on gap junctions (O’Keefe, 2006). Sharp wave-ripples in the hippocampus-entorhinal cortex are important for memory consolidation, which involves transferring memories from the hippocampus to the neocortex for long-term storage. Selective elimination of sharp wave-ripples by electrical stimulation of the ventral hippocampus during post-training periods was found to impair spatial memory in rats (Girardeau et al, 2009).

1.4.1.2. Cortex

While the somatosensory cortex generally shows desynchronized, low amplitude activity in the awake state, network oscillations known as delta waves (0.5-4Hz) emerge during sleep. Cortical EEG oscillations seen during sleep consist of polymorphic waves
resulting from three separate oscillations, slow cortical oscillations (<1 Hz), cortical delta oscillations and thalamic delta oscillations (Amzica and Steriade, 1998).

Slow oscillations occur first in the sleep cycle, and are synchronous throughout various areas of the cortex. They reflect membrane potential oscillations between depolarized “UP” and hyperpolarized states. Slow oscillations appear as a “K” complex on the EEG, with a sharp negative deflection followed by a dome-shaped positive potential. The depolarized phase is the result of excitatory and inhibitory synaptic potentials, while the hyperpolarized phase may be mediated by slow Ca2+-dependent K+ currents. Blockade of NMDA receptors reduces the duration of the depolarized state, and thus leads to shorter cycles (higher frequency oscillations). Acetylcholine blocks the hyperpolarization phase and eliminates slow oscillations (Amzica and Steriade, 1998).

Another component of the sleep EEG is cortical delta oscillations. Cortical delta oscillations rely on intrinsically bursting neurons, which respond to a depolarizing current (such as during the UP state of slow oscillations) with bursts of action potentials at a frequency of 3-4Hz. There is no evidence for synchronization between intrinsically bursting neurons from different regions of the cortex. These tend to be localized, short-lasting, and contribute to the polymorphic character of the sleep EEG but do not provide a regular rhythmic oscillation (Amzica and Steriade, 1998).
The third oscillation contributing to the sleep EEG is a rhythmic, clock-like thalamic delta oscillation (1-4Hz) created by the interplay between low-threshold transient Ca2+ currents (Ih) and hyperpolarization-activated cation currents (Ih). Thalamic delta cycles are believed to reach the cortex at more negative membrane potentials (Amzica and Steriade, 1998).

1.4.2. In vitro Studies

1.4.2.1. Synaptic Plasticity

Neurophysiological changes in Rett mice have been examined predominantly in vitro. Synaptic plasticity is an essential form of learning and memory whereby neuronal connections are either strengthened or weakened as a result of previous synaptic activity patterns (http://en.wikipedia.org/wiki/). Synaptic plasticity is impaired in symptomatic MeCP2-deficient mice which may in part explain defects in spatial learning (Stearns et al, 2007). MeCP2<sup>308/Y</sup> mice take longer to locate a hidden platform, despite intact motor function, in the Morris Water maze task, a test of spatial learning ability. When the platform was removed, these mice spent an equal amount of time in all quadrants whereas controls spent the majority of their time in the quadrant where the platform had previously been (Moretti et al, 2006). MeCP2-null mice could not be assessed using this test as motor impairments would confound results. Female MeCP2-heterozygous mice did not show
impaired spatial navigation in a swimming task, however, both Mecp2-null and Mecp2-heterozygous mice performed poorly on novel object recognition, another hippocampal-dependent learning task (Stearns et al, 2007).

Studies on Mecp2-null and Mecp2^{308/Y} hippocampal slices revealed impaired long term potentiation (LTP) in the schaffer-collateral pathway; impaired LTP was also found in the motor cortex and primary sensory cortex of Mecp2^{308/Y} mice (Asaki et al, 2006; Moretti et al, 2006). LTP is a form of synaptic plasticity whereby simultaneous stimulation of neurons results in long-lasting enhancement (ranging from hours to days) of synaptic transmission between these neurons. One frequently used paradigm to induce LTP involves stimulating presynaptic fibers at high frequencies (around 100Hz) for short periods of time and recording intracellular responses of postsynaptic neurons. The postsynaptic neuron’s response to single pulse stimulation of presynaptic fibers is significantly enhanced following high frequency stimulation (HFS) (http://en.wikipedia.org/wiki/). In the hippocampus, LTP is commonly assessed by stimulating the schaffer-collateral pathway and recording from pyramidal neurons of the CA1, although LTP can be assessed in other hippocampal pathways as well. One molecular mechanism for LTP involves NMDA-mediated regulation of synaptic receptors. Repetitive activation of NMDA receptor channels has been found to increase the number of AMPA receptors added to the postsynaptic membrane via exocytosis (http://en.wikipedia.org/wiki/). Theta-burst stimulation, a more physiological form of synaptic plasticity induction in which short trains
of 5Hz stimulation are applied to presynaptic fibers, failed to induce LTP in the CA1 of symptomatic MeCP2-null mice (Asaka et al, 2006).

Long term depression (LTD) was absent in symptomatic MeCP2-null males and impaired even in asymptomatic, 4-6 week old Mecp2<sup>308/Y</sup> males (Asaka et al, 2006; Moretti et al, 2006). Long term depression is an activity-dependent reduction in the strength of neuronal synapses lasting hours or longer. LTD can be induced in the hippocampus by low frequency stimulation (around 1 Hz) of the schaffer-collateral pathway. LTD may rely on NMDA receptor activation and involves a reduction in postsynaptic receptor density (http://en.wikipedia.org/wiki/). These findings suggest that the MeCP2-deficient brain remains in an immature state, where it is not able to form stable connections essential in learning. Altered expression of NMDA NR2 receptor subunits may underlie changes in synaptic plasticity in MeCP2-deficient mice (Asaka et al, 2006).

1.4.2.2. Excitation and Inhibition in the Rett Brain

Despite the severe phenotype of MeCP2-null mice, no gross neurophysiological abnormalities have been observed. Dendritic spine density is reduced, whole brain volume is reduced, and decreased neuronal size results in denser neuron packing in the MeCP2-null brain (Belichenko et al, 2008). However, other morphological features are preserved and neurons of the MeCP2-null brain display seemingly normal functional properties. One study examined layer 5 pyramidal neurons in somatosensory cortex slices incubated in a
modified artificial cerebrospinal fluid solution (Dani et al, 2005). In Mecp2-null slices, spontaneous firing rates were decreased approximately four fold for symptomatic-aged mice. Even at younger ages, a two fold reduction in the spontaneous firing rate was observed. The intrinsic electrical properties of these neurons were found to be normal. Rather, there appeared to be reduction of excitatory synaptic input (of approximately 58%) onto L5 pyramidal cells, and increased inhibitory synaptic input. This decrease in excitatory synaptic input may be in part be caused by decreased quantal release of the glutamate neurotransmitter (15% less than control levels) (Dani et al, 2005).

Insulin growth-like factor 1 (IGF-1) is a neurotrophic factor which elevates excitatory postsynaptic currents (EPSCs) significantly (Tropea et al, 2008). When administered to Mecp2-null mice from the age of 2 weeks onwards, improvements in phenotypic outcome resulted. Median lifespan increased from 60 to 90 days. When tested at 8 weeks of age, locomotion was increased, and heart rate and breathing variability improved, although not to wild-type levels. There was also an increase in brain size in IGF-1 treated Mecp2-null mice and improvement of the synapse. Levels of postsynaptic density protein 95 (PSD-95), a scaffold protein involved in synapse maturation and plasticity, were increased and the number of dendritic spines also increased (Tropea et al, 2008). These studies suggest that there is an imbalance between synaptic excitation and inhibition in the Rett brain, and that drugs which correct this imbalance, such as IGF-1 which increases excitatory synaptic drive, may be beneficial in the treatment of RTT.
1.4.2.3. Hippocampal CA3 circuitry

Zhang et al (2008) examined hippocampal network activity in vitro in the MeCP2-null brain. In the CA3 subregion of the hippocampus, spontaneous rhythmic field potentials (SRFPs) occur, with a frequency between 0.5-4Hz. SRFPs are generated by the interplay of GABA-A mediated IPSPs in CA3 pyramidal neurons and EPSPs on local inhibitory neurons. Zhang et al (2008) found a 50% decrease in the frequency of SRFPs from 1.32 Hz in WT to 0.65 Hz in MeCP2-null mice. When a low dose of bicuculine, a GABA-A receptor antagonist, was administered to the CA3 region, multiple spike waveforms were generated, suggesting the hippocampal circuitry entered a state of hyperexcitability.

In vitro studies collectively suggest that there is an imbalance between excitation and inhibition in the MeCP2-deficient brain. Excitation appears to be decreased, perhaps due to decreased spine density in the brain or decreased quantal release of excitatory neurotransmitters, and inhibition is increased. At the network level this leads to reduced spontaneous activity in principal cells. Drugs that increase excitation, such as IGF-1, may alleviate many of the symptoms of RTT in mice and increase median life expectancy (Tropea et al, 2008). Paradoxically, the MeCP2-deficient brain appears prone to hyperexcitability such that slight tweaking of this balance, for instance a low dose of bicuculine, is able to promote hypersynchronous discharges (Zhang et al, 2008).
1.4.3. *In vivo* studies

1.4.3.1. Seizures in Rett mice

Only one study examined the EEG of *Mecp2*-deficient mice (Shahbazian et al, 2002). A subdural, bilateral recording of the frontal and parietal cortex of *Mecp2*\(^{308/Y}\) mice was taken. Several *Mecp2*\(^{308/Y}\) mice displayed spontaneous myoclonic seizures. However, no additional information on network activity was provided.

1.5. Rationale

1.5.1. EEG

Seizures remain poorly understood in RTT. Although Rett girls harbour similar genetic abnormalities, such a varying profile of EEG abnormalities and seizure patterns is observed (Glaze, 2005). Rett girls are often heavily medicated, have different *MECP2* mutations, X-chromosome inactivation patterns, and environmental exposures, all factors that can affect the phenotype outcome of RTT (INTRODUCTION 1.1.4). Therefore, a more controlled study on animal models is needed to study the EEG of RTT and to better understand the consequences of MeCP2-deficiency in the brain. The behaviour of *Mecp2*-deficient mice has been well characterized. Morphology and neurochemistry studies have also been conducted on these mice. Only one study, however, has looked at the EEG of
Mecp2-deficient mice (Shahbazian et al, 2002). Their examination was shallow, and the only reported finding was the presence of myoclonic seizures. The implantation method used subdural electrodes and therefore EEG signals were not localized. The researchers only examined cortical structures; deeper brain structures such as the hippocampus were not examined. A detailed examination of the EEG of Mecp2-deficient mice is necessary to elucidate the EEG patterns associated with MeCP2 loss in the brain.

1.5.2. Hippocampus and somatosensory cortex

The hippocampus is a well defined structure with a characteristic flow of information (INTRODUCTION 1.4.1). Hippocampal functionality is impaired in all Mecp2-deficient models of RTT as assessed by the Morris Water Maze and novel object recognition tasks (Moretti et al, 2006; Stearns et al, 2007). Deficits in hippocampal learning may be linked to changes in hippocampal theta oscillations. Levels of ACh in the basal forebrain of Rett girls are reduced (Wenk et al, 1997), which would predict a decrease in the amplitude of hippocampal theta oscillations. These observations make the hippocampus an attractive area of investigation in vivo. Another reason to study the hippocampus is that in vitro studies have looked at hippocampal network activity, and it would be worthwhile to determine if these findings are extended in vivo. The CA1 region was chosen because EEG theta oscillations are prominent and neuron packing is denser resulting in a higher amplitude signal (O’Keefe, 2006). The somatosensory cortex site was chosen to give a
general cortical signal. Also, in vitro studies have examined activity in the pyramidal cells of the somatosensory cortex (Dani et al, 2005).

1.5.3. Mecp2-heterozygous and Mecp2-null mice

Mecp2-null mice have traditionally been used for most studies of RTT. However, it is believed that the true counterpart of Mecp2-null mice is infants with a condition called congenital encephalopathy which results from MECP2 mutations in males (Schule et al, 2008). These males have a far more severe condition than Rett girls and die prematurely (usually before the age of 3) from respiratory problems. Unlike Rett girls, males with congenital encephalopathy do not show stereotypies, such as hand wringing, and have apneic episodes in sleep and wakefulness in contrast to Rett girls who have normal breathing during sleep (Schule et al, 2008). Therefore, it seems appropriate to use both Mecp2-null and Mecp2-heterozygous mice in the study of RTT and have all bases covered.

1.5.4. Chronic Recordings

RTT follows a characteristic progression of behavioural symptoms and EEG abnormalities in Rett girls. Seizures are considered a later symptom of RTT. EEG abnormalities initially worsen, but may actually improve in later stages (INTRODUCTION 1.1.1). Therefore, in order to determine whether or not a similar EEG progression occurs in Mecp2-deficient mice, mutant mice should be recorded regularly from the age of symptom
onset until death. Another advantage of chronic recordings is that mice habituate to the electrode setup allowing for the observation of relaxed, immobile behavioural states.
2. OBJECTIVE AND HYPOTHESES

2.1. Main Objective and Hypothesis

The main objective of this thesis is to describe the baseline hippocampal and cortical EEG of \textit{Mecp2}-heterozygous and \textit{Mecp2}-null mice, using intracranial electrodes, over the time course of the symptomatic stages of RTT. A secondary objective will be to address whether baseline EEG abnormalities and or seizures seen in Rett mice are amenable to pharmacological treatment.

The overall hypothesis is that a deficiency of MeCP2 in the brain will cause baseline EEG abnormalities in the hippocampal and somatosensory cortex, as well as an increased propensity to develop seizures. Furthermore, these abnormalities will be more severe in \textit{MeCP2}-null mice, which completely lack MeCP2, compared to the \textit{Mecp2}-heterozygous group, in which MeCP2 levels are approximately 50\% of wild-type levels in the brain.

2.2. Specific Hypotheses

More specifically, the experiments of this thesis will aim to test the following hypotheses:

1. Hippocampal theta network oscillations will show impairments in frequency and amplitude in accordance with findings that Rett mice show impairments in hippocampal dependent tasks and have reduced intrinsic network activity (Stearns
et al, 2007; Zhang et al, 2008). Exploratory theta fragments will be shorter as Mecp2-deficient mice are hypoactive.

2. Epileptic seizures will occur in Mecp2-deficient mice. Specifically, generalized convulsive and myoclonic seizures should be seen (Glaze, 2005; Shahbazian et al, 2002). EEG abnormalities will show a progression similar to the stages in Rett girls.

3. Sharp wave discharges will be occur more frequently during LIA reflecting a brain prone to hypersynchronous discharges (Glaze, 2005; Zhang et al, 2008).

4. Delta oscillations during slow wave sleep will have a normal frequency distribution in Mecp2-deficient mice.
3. METHODS

3.1. Animals

Subjects were generated by crossing female Mecp2-heterozygous mice purchased from Jackson Laboratory (Mecp2tm1.1Bird, Jackson Laboratory, Bar Harbor, ME) with male wild-type mice as described previously (Asaka et al., 2006; Jugloff et al., 2006). Mice were maintained on a C57BL/6 background. Mice were singly caged (after implantation), kept on a 12-hour light cycle (lights on 6am to 6pm) with temperature held constant at 23°C. Mice were supplied with water and standard commercial chow ad libitum.

3.2. Drugs

Tribromoethanol (Avertin) was prepared by dissolving 2.5g of 2,2,2-tribromoethanol with 5mL of tert-amyl alcohol, then diluted to 2.5% in distilled water. The solution was stored in a 4°C refrigerator. Previous studies have found Avertin to be effective at inducing surgical anesthesia in small laboratory animals (Norris and Turner, 1983; Green CJ, 1975). Avertin was used to anesthetize wild-type in preparation for the implantation procedure. An inhalational isofluorane solution was used to maintain surgical anesthesia in Mecp2-deficient mice. Ketoprofen (Anafen) solution was diluted to a concentration of 10mg/mL and was used after surgery as a preemptive analgesic. Ethosuximide, a T-type Ca2+
channel blocker known to block absence seizures in humans and rodents (Goren and Onat, 2007), was prepared fresh by dissolving 5mg in 1mL of distilled water, and then diluted to a final concentration of 1mg/mL. Aforementioned drugs and chemical compounds were purchased from Sigma Aldrich (Oakville, ON, Canada).

3.3. **In vivo Experiments**

The methods described in **METHODS** (3.3.1.) and (3.3.2.) have been published in Wu et al., 2008.

### 3.3.1. Electrode Construction

Each electrode was assembled using a recording wire made from polyimide-insulated stainless steel (outer diameter 0.25mm and inner diameter 0.21mm, Plastics One, Roanoka, VA, USA), soldered to a connecting male pin. The resistance of the stainless steel wire was $0.68 \pm 0.03\Omega$ when measured from a piece 20mm in length. The wire was scratched at one end to remove the insulating layer for soldering. The connecting pin was 6mm in length and was cut from a 21-guage stainless steel tube (Plastics One). A small cut was made at one end for better contact between the connecting pin and epoxy to be applied. A soldering liquid (Soldering Liquid Flux, Certanium Alloys and Research Company, Cleveland, OH, USA) was used to connect the recording wire to the connecting pin. Recording electrode assemblies were cleaned overnight in 80% ethanol. A 5 x 5mm square
was cut from the curved portion of a plastic weighing dish (VWR International, Mississauga, ON, Canada) and used as the base of the electrode cap. Three small holes (0.3mm diameter) were made in the plastic base, with their positions corresponding to the stereotactic coordinates of the intended recording sites. The recording electrodes were put through the base, and fastened by pipetting dental cement on top of the base, around the recording electrodes. The bottom of the plastic base was clean, ensuring good contact with the skull during electrode implantation. After the dental cement hardened overnight, the recording wires were cut to match the depths of the recording sites. The assembled electrodes were cleaned with 75% alcohol and stored in a sterilized glass bottle prior to implantation. The total weight of the electrode assembly was around 100 mg.

3.3.2. Surgery and Electrode Implantation

All surgical instruments were sterilized prior to use and all injections were made using a 30-gauge needle. Wild-type animals were anesthetized with Avertin (intraperitoneal [i.p.], 2.5%, 0.2mL/10g). Avertin reliably induced surgical level anesthesia in mice and did not result in any fatalities in wild-type mice. However, several MeCP2-deficient mice did not survive or recover from surgery under Avertin-induced anesthesia. For this reason, the inhalational anesthetic isoflurane was used in the implantation of mutant mice. Mutant mice were placed in a glass chamber with an isoflurane concentration of 5% for anesthesia induction. Once surgical anesthesia was reached, animals were placed
onto a stereotaxic frame and held in place by a mouse adaptor (Kopf Instruments, CA, USA). The incisor bar was positioned 3mm below the interaural zero reference point. For MeCP2-deficient mice, a nose cone positioned over the snout supplied a mixture of isoflurane and oxygen (1-3% isoflurane) to maintain surgical anesthesia. The mouse’s head was swabbed with antiseptic betadine solution, a strong microbial which prevent infection, before the skin on top of the head was incised with a scalpel and gently separated with forceps. Three small holes (0.5mm diameter) were drilled in the skull at specific stereotactic coordinates, and standard Q-tips were used to absorb excess blood. The EEG recording electrodes were positioned in the hippocampal CA1 area (Bregma -2.3mm, lateral 1.7mm and depth 2.0mm) and contralateral parietal cortex (Bregma -0.8m, lateral 1.8mm and depth 1.5mm). A reference electrode was placed near the cortical electrode (Bregma -3.8mm, lateral 1.8mm, depth 1.5mm). The reference electrode was roughly equidistant from the two recording electrodes, creating an isosceles triangle between the electrodes. The position of the reference electrode was chosen for technical reasons, specifically, to minimize the surface area of the electrode cap assembly. The electrode assembly was then lowered on top of the skull and secured into place using a quick bonding, cyanoacrylate-based glue (Insta-cure+, BSI Adhesives, CA, USA). Although different anesthetics were employed in the surgery of wild-type and MeCP2-deficient mice, animals were allowed to recover at least a week before EEG assessment, ensuring that anesthetic effects on brain activity and/ or behavior were negligible. Brain slices were fixed post-mortem to confirm
that recording electrodes had reached their target location. These results have been published in Neurobiology of Disease (D’Cruz et al, 2010).

3.3.3. EEG recordings

EEG recordings were monopolar, and thus reflected the difference in electrical activity between a recording site in the hippocampus or somatosensory cortex, and the reference electrode. An EEG signal was recorded from the CA1 hippocampus and somatosensory cortex using two extracellular amplifiers with extended head-stages (Model-300, AM Systems Inc., WA, USA). The head stages were secured 10cm above the animal’s housing cage and connected to the electrode assembly using soft wires with connecting female pins (gold wire contacts, Fine Scientific Tools Inc., Vancouver, Canada) at the end. The female pins of the recordings wires were connected to the male connecting pins of the electrode cap assembly while restraining the mouse by the tail. No anesthetics were used in this process.

EEG signals in the 0.05-1,000Hz frequency band were recorded and amplified 1000x before digitization (digitization rate of 60kHz; Digidata 1300, Molecular Devices, CA, USA). A 60Hz notch filter was applied to reduce noise caused by electrical interference. Data acquisition and storage were performed with pCLAMP software (Molecular Devices, version 9.0). Mice were continuously monitored during EEG recording sessions and
comment tags were time-locked to behavioural events (see figure 4). Each recording session lasted 30 minutes. Consecutive recordings were often taken in the same mouse in order to observe all behavioural states and allow for habituation to the setup.
Figure 4. Recording setup for EEG assessment. Recording wires are connected to the electrode assembly on the mouse. An EEG signal is simultaneously recorded from the CA1 hippocampus and somatosensory cortex. The mouse is continuously monitored and comment tags are added to the data file to denote behavioural state.
Data Analysis

For data analysis, original files were reduced 10x to a more manageable size and bandpassed filtered (Bessel 8-pole) 0.667-200Hz. The sampling rate of the new files was 6 kHz. The Nyquist Sampling Theorem states that for a signal to be properly sampled, it should not contain frequencies above one-half of the sampling rate. Biological EEG signals have an upper frequency of limit of ~500Hz. According to the Nyquist Sampling theorem, a sampling rate of at least 1000Hz should be used. In practice, sampling rates of at least five times the signal frequency are used. The 6 kHz sampling rate of the new data files was well above this frequency. A high pass filter was used to remove slower oscillations resulting from movement artifact; 0.667 Hz was the lowest high pass filter value that could be applied at the sampling rate of the reduced file. A 200Hz low pass filter was used to remove biologically irrelevant noise to give a cleaner trace. While EEG oscillations of up to 500Hz may occur in the brain, the thick size (150um) of the recording electrode prevented detection of EEG oscillations above 200Hz. Behavioural states were classified into four categories: sleep, immobile-awake, consummatory (eating and grooming) and exploration (foraging, sniffing, moving and rearing). The EEG frequency profile was assessed during each behavioural state using power spectrum analysis.

Power Spectrum Analysis
Power spectrum analysis produces a graphical output of the frequencies contributing to an EEG signal and their relative contribution (Smith, 1997). Frequency is displayed along the x-axis and amplitude or power along the y-axis. Power spectrum analysis uses a fast-fourier transform (FFT) algorithm to calculate the discrete fourier transform (DFT) of an EEG signal. DFT decomposes an input sequence (time domain) into sinusoidal functions of different frequencies (frequency domain).

An input sequence \( x[i] \) with \( N \) points can be synthesized from \( N/2 \) cosine functions as shown in equation 1. Input sequence length \( N \) is defined by the user, and is typically a factor of 2, such as 1024 \( (2^{10}) \) or 4096 \( (2^{12}) \). The frequency of each cosine function is given by \( k \), which is the number of cosine cycles completed in \( N \) points, and the amplitude of each cosine function is denoted by \( X[k] \).

\[
\frac{N}{2} x[i] = \sum_{k=0}^{\frac{N}{2}} X[k] \cos(2\pi k i/N)
\]

Equation 1. The input signal can be synthesized from \( N/2 \) cosine functions. Values for \( i \) run from 0 to \( N-1 \).

The DFT is simply the inverse of equation 1 and can be calculated in several ways (Smith, 1997).

The first method is DFT by simultaneous equations. According to equation 1, the value of the input sequence at any point \( i \) is equal to the sum of the scaled cosine functions at point \( i \).
The amplitude of the cosine functions $X[k]$ is unknown and for each of the $N$ points an equation with $N/2$ unknown variables will be generated. These simultaneous equations can then be solved using Gaussian elimination. Although intuitive, this first method is computationally intensive and rarely used. The second method is DFT by correlation. Each cosine function is multiplied by the input sequence $x[i]$ and the points of the resulting function are summed to give an amplitude value (see equation 2). If a particular cosine wave is not present in the input sequence, the resulting function should sum to zero. An amplitude of zero will be assigned to that particular cosine function.

$$X[k] = \sum_{i=0}^{N-1} x[i] \cos(2\pi ki/N)$$

Equation 2: The amplitude of cosine waves is found by multiplying the input signal by each cosine function. Values of $k$ run from 0 to $N/2$.

The third method for calculating the DFT is fast fourier transform (FFT). There are several FFT algorithms, none of which will be discussed here. FFT is typically hundreds of times faster than the other methods of calculating DFT (Smith, 1997).

Power spectrum analysis is a convenient method for determining the prominent frequencies in an EEG trace. The average frequency of a particular waveform can be calculated manually, measuring the interval between peaks or troughs. However, this method is time consuming and does not reflect all of the frequencies present in the signal.
For instance, exploratory theta sequences are often composed of multiple theta frequencies and gamma oscillations are often superimposed atop theta waves. Power spectrum analysis of an exploratory theta epoch produces a single broad peak in the theta range and a smaller second peak in the gamma range, whereas manual theta frequency detection would produce a single value in the theta range. Power spectrum analysis is thus far more informative.

A couple of problems are associated with modeling an EEG signal with sinusoidal waveforms. EEG input signals are considered aperiodic because they are finite in length, and therefore do not repeat infinitely. However, the sinusoidal functions that collectively model the input sequence are periodic by nature. In order to get around this discrepancy the input sequence is treated as a single period, and for computational purposes identical signals are assumed to repeat to the left and right of the window being analyzed. Another problem associated with modeling the input sequence by sinusoidal functions is that biological waveforms are not sinusoidal. While theta and gamma oscillations can be approximated very closely by sinusoidal waveforms, cortical delta frequency oscillations are largely irregular in waveform. Thus analyzing delta frequency by FFT is likely associated with larger errors than its application to the theta or gamma activities.

*Theta*
Hippocampal theta epochs were first identified manually as a sinusoidal waveform in the 6-12Hz range. To calculate the average theta frequency in each animal, theta epochs of at least 5s duration were used. These ~5s EEG segments were fast-fourier transformed, with 4096 points per window and 50% window overlap to produce a power spectrum plot with 0.41 Hz resolution. The frequency of a given theta epoch was taken as the frequency at which the dominant peak occurred on the power spectrum plot. A minimum of 11 epochs per animal was used to calculate average theta frequency. Theta frequency averages were then averaged in each group to produce a group mean. Mice with 10 or less exploratory theta epochs were not used for statistical comparison between groups. The average length of theta segments was found by averaging the total length of all the exploratory segments in the mouse. Transitions into and out of EEG theta were usually distinct so that the onset and termination of hippocampal theta rhythm could be detected visually. Individual theta segment length averages were averaged across members of the same genetic group.

**Delta**

Slow wave sleep (SWS) segments were characterized by a hippocampal LIA rhythm, marked with frequent sharp wave events, and a cortical trace displaying prominent, 0.5-4Hz sinusoidal waves. SWS segments were manually detected on the EEG. Peak delta frequency was calculated using power spectrum analysis of ~30s cortical slow wave epochs. For consistency, the same parameters employed in theta analysis were used. Sample
windows were 4096 points with 50% window overlap and a spectral resolution of 0.41Hz. Power spectrum plots were averaged across SWS segments, and then across mice from the same group to produce a frequency distribution plot. A minimum of 10 segments per animal was used to calculate average peak frequency. Hippocampal traces of the SWS segments were analyzed in the same manner.

*Sharp waves*

Sharp waves have been reported to occur in all of the LIA-associated behaviours. With the intent of keeping a consistent behavioural state for all sharp wave analyses, only SWS segments marked for delta wave analysis were used. Sharp wave events were recognized manually and were required to meet certain criteria of inclusion. Sharp waves were identified as sharp downward deflections, with a duration of less than 100ms, and an amplitude at least 1.5 SD above baseline variation. Waveforms that met these criteria were not included if they were contaminated with oscillations at other frequencies (not including ripples, which are known to be superimposed atop sharp waves). The average interval between sharp wave events was calculated in each SWS segment to produce an average inter- sharp wave interval. Inter- sharp wave intervals were then averaged across groups.

### 3.4. Statistics

Most group comparisons involved a simple statistical test, the student’s t-test, which was performed using excel. For all statistical tests, a two-tailed, unpaired comparison was
made. Unless otherwise noted, data are presented as mean values ± the standard error of the mean (SE). P values of less than 0.05 were deemed significant in all statistical tests.

4. RESULTS

4.1. The frequency of hippocampal theta oscillations is reduced in MeCP2-deficient mice. The average length of exploratory theta segments does not differ among groups.

The hippocampal theta rhythm of MeCP2-deficient mice was expected to display reduced amplitude and frequency on the basis that these mice show impairments in hippocampal-dependent learning tasks (Moretti et al, 2006; Stearns et al, 2007) and a reduction in the frequency of spontaneously occurring CA3 network oscillations (Zhang et al, 2008). Although behavioural studies on the MeCP2-deficient mice have found these mice to be hypoactive (Chen et al, 2001; Guy et al, 2001), especially in the MeCP2-null group, exploration appeared normal in mutant mice. MeCP2-deficient mice engaged in normal exploratory behaviours such as sniffing, rearing, moving, and foraging, and these were accompanied by EEG theta oscillations in the CA1 hippocampus. Visually, the hippocampal trace of MeCP2-heterozygotes showed a normal theta-range sinusoidal oscillatory pattern during exploration while the somatosensory cortex trace displayed low
amplitude, desynchronized activity (figure 5a). Likewise, the EEG activity of Mecp2-null mice was comparable to controls in the exploratory state (figure 6a).

To calculate theta frequency, the peak frequencies of individual exploratory theta epochs were determined by power spectrum analysis and then averaged across recording sessions for each animal. Individual averages were then averaged within groups in order to calculate the mean theta frequency of Mecp2-heterozygotes (n=8), their aged-matched controls (n=5), Mecp2-null mice (n=5) and their wild-type counterparts (n=3). In all groups, power spectrum analysis of individual hippocampal theta segments revealed a dominant peak in the theta range (6-10Hz). Peak theta frequencies were fairly consistent across theta epochs from the same animal and in general showed little within-group variation. The Mecp2-heterozygous group showed the greatest variation in theta frequency (standard deviation 0.43 compared to 0.08 in their control group, 0.15 in the Mecp2-null group, and 0.10 in the null-control group). The average theta frequency was significantly lower in 9-14 month old Mecp2-heterozygous mice compared to controls (8.2 Hz vs. 9.1 Hz in wild-types, p < 0.01, figure 5b). In the two Mecp2-heterozygotes recorded beyond the age of 14 months, J2 and AB07-2, the theta frequency calculated during the 9-14 month age window was compared to the theta frequency average obtained from recordings after 14 months. Peak theta frequency did not appear to change significantly with age in these two mice. J2 showed a slight decrease in average theta frequency overtime, from 7.4 to 7.2 Hz (p = 0.45), while the average peak theta frequency of AB07-2 increased marginally from 8.1 to 8.2 Hz.
Mecp2-null mice and their age-matched controls were recorded from 60 to 120 days or until the age of death. Given the limited time window, age-related changes were not examined in these mice. Mecp2-null mice, surprising, showed a normal profile of theta activity (figure 6a) although theta-frequency was reduced ~20% from 8.9Hz to 7.1 Hz (p < 0.01, figure 6b).

The overall amplitude of hippocampal EEG signals was highly variable between mice of the same group, and fluctuated from one recording session to the next in the same animal. For this reason, hippocampal theta amplitude was not compared between groups.

The average length of exploratory theta segments did not significantly differ between Mecp2-heterozygous mice and their controls, or between Mecp2-null mice and their controls (figure 5c, 6c). Male wild-types (figure 6c) appeared to explore ~10s longer than female wild-types. This finding may be attributed to differences in age between male controls (60-120 days) and female controls (9-14 months). The above results partially supported the stated hypothesis that theta oscillations would be severely impaired in Mecp2-deficient mice, although only slight reductions to the frequency of hippocampal theta oscillations were found.
Figure 5. Comparison of hippocampal theta between Mecp2-heterozygotes and controls during exploration. A, Representative 15s hippocampal (hipp) and cortical (cort) EEG traces of Mecp2-heterozygous and wild-type mice during exploration (top) with 4s segments blown-up (middle) and analyzed using power spectrum analysis (bottom). B, C, Histograms depicting average exploratory theta frequency and length respectively in Mecp2-heterozygous and control groups. * , p < 0.01.
Figure 6. Comparison of hippocampal theta between Mecp2-null and control mice. 

A, Representative 15s hippocampal (hipp) and cortical (cort) EEG traces of Mecp2-null and wild-type mice during exploration (top) with 4s segments blown-up (middle) and analyzed using power spectrum analysis (bottom). Solid lines below top cortical trace of Mecp2-null mouse denote epileptiform-like discharges. 

B, C, Histograms depicting average theta frequency and length respectively in Mecp2-null and control groups. * , p < 0.01.
4.2. Spontaneous slow spike and wave discharges are observed in the somatosensory cortex of several *Mecp2*-deficient mice. EEG abnormalities do not show a staged progression.

Baseline network activity was largely preserved in *Mecp2*-deficient mice. During exploration, the only EEG alteration seen was a slight reduction in the frequency of hippocampal theta oscillations in *Mecp2*-deficient mice (RESULTS 4.1). The other behavioural states examined, immobile-awake and sleep, also showed normal EEG patterns. During the immobile-awake behavioural state, the cortical EEG of *Mecp2*-heterozygous mice displayed low amplitude, desynchronized activity while the hippocampal trace showed typical large amplitude, irregular activity (LIA) (figure 7a). Power spectrum analysis of EEG segments from the immobile-awake state in *Mecp2*-heterozygotes (n=3) and controls (n=3) showed a similar frequency distribution between groups in the hippocampus and somatosensory cortex (figure 7c). Power spectrum plots of hippocampal traces were nearly parallel although the overall amplitude appeared lower in *Mecp2*-heterozygotes relative to controls. As expected, there was a broad spread across the lower frequencies (Lawson and Bland, 1993). The cortical EEG showed similar profiles in both groups. Finally, the sleep behavioural state also displayed normal EEG patterns which are considered in more detail in RESULTS 4.1.3, 4.1.4.
Figure 7. EEG comparison between *Mecp2*-heterozygotes and wild-type mice in the immobile-awake behavioural state. **A, B:** Representative 12s EEG traces of CA1 hippocampus (hipp) and somatosensory cortex (cort) in *Mecp2*-heterozygotes (A) and wild-type mice (B) during behavioural immobility. **C:** Unnormalized power spectrum plots of immobile-awake segments from the hippocampus and cortex of *Mecp2*-heterozygotes (black line) and wild-type mice (light gray).
While EEG patterns of *Mecp2*-deficient mice generally matched those seen in wild-types, the majority (8 out of 12) of 9-14 month old *Mecp2*-heterozygotes displayed abnormal cortical spike and wave discharges (SWD). SWD events occurred in the immobile-awake behavioural state, or sometimes interrupting an exploratory theta segment, but were not seen during actual theta or slow wave sleep in these mice. SWD events were present in multiple recording sessions from the same animal, sometimes months apart. Representative EEG traces from *Mecp2*-heterozygote OCI 52 are shown in figure 8a. The hippocampus shows irregular, large amplitude (LIA) activity while the cortex shows a rhythmic spike and wave discharge pattern, expanded below. Power spectrum analysis of cortical discharge segments revealed a clear peak in the theta-range (6-9Hz). SWD events took on a slightly different profile in each animal as illustrated in table 1. They were typically between 1-2 seconds in duration, with an amplitude 2.5x baseline and frequency in the theta range, between 6-9Hz. The incidence varied greatly, from an average of four to thirteen events per hour. The appearance of spontaneous SWD events appears to be age-dependent. A couple of mice clearly did not show SWD events in earlier recordings but did so later on. For instance, AB07-2 did not show any oscillations when recorded over a three hour period at the age of 8 months, but showed prominent oscillations when recorded four months later.

Ethosuximide, an anti-absence drug which works as a T-type Ca2+ channel blocker, was administered i.p. to a subset of *Mecp2*-heterozygous mice displaying SWD events, at a
dosage known to block seizures in absence rodent models, 100mg/kg. The drug greatly reduced the incidence of SWDs from an average of 8.5 to 1 episodes per hour (figure 8b). Only one mouse, J2, failed to respond to administration of ethosuximide.
Figure 8. Cortical discharges are seen in *Mecp2*-heterozygous mice.  

**A**: 10s representative EEG traces from a single recording session of *Mecp2*-heterozygote OCI 52 are shown (top). Cortical discharge segments are expanded below (middle) and analyzed using power spectrum analysis (bottom).  

**B**: Histogram comparing SWD incidence before and after administration of 100mg/kg ethosuximide. *, p < 0.01.
Table 1. Characteristics of cortical spike and wave discharges seen in Mecp2-deficient mice. * indicates Mecp2-null genotype; all other mice are Mecp2-heterozygotes.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Average Frequency</th>
<th>Average Duration</th>
<th>Amplitude (x baseline)</th>
<th>SWD/ hour</th>
<th>Ethosuximide (occurrence/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R617</td>
<td>9.1/8.9</td>
<td>1.5</td>
<td>2.5</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>R607</td>
<td>8.6/8.1</td>
<td>1.3</td>
<td>2.5</td>
<td>5.2</td>
<td>1</td>
</tr>
<tr>
<td>J2</td>
<td>7.9/7.4</td>
<td>1.1</td>
<td>3</td>
<td>11.1</td>
<td>10</td>
</tr>
<tr>
<td>AB07-2</td>
<td>8.4/8.0</td>
<td>1.2</td>
<td>3.2</td>
<td>12.2</td>
<td>2</td>
</tr>
<tr>
<td>Oci 11</td>
<td>7.2/6.5</td>
<td>1.3</td>
<td>2.4</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Oci 52</td>
<td>8.0/7.6</td>
<td>1.5</td>
<td>2.6</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>JL-8</td>
<td>8.0/8.1</td>
<td>1.4</td>
<td>2.4</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>JL-5</td>
<td>8.6/7.7</td>
<td>0.9</td>
<td>2.5</td>
<td>8.6</td>
<td>0</td>
</tr>
<tr>
<td>*08-551</td>
<td>6.8/6.5</td>
<td>1.8</td>
<td>3.2</td>
<td>11.6</td>
<td>0</td>
</tr>
</tbody>
</table>
Of the four *Mecp2*-null mice recorded, only one showed rhythmic SWD events (figure 9). The SWD events recorded from this subject showed a similar profile to those seen in *Mecp2*-heterozygotes in terms of amplitude, frequency, duration and incidence (table 1). However, SWD events occurred in all behavioural states in *Mecp2*-null mouse 08-551. Figure 9 shows SWD events occurring during slow wave sleep (upper trace) and during a transitory state between exploration and immobile-awake (lower trace) in 08-551. Brief SWD episodes were also seen during exploratory theta and are marked by a solid bar in figure 6a. Another distinguishing feature is that both hippocampal and cortical channels are involved, whereas SWDs are limited to the somatosensory cortex in *Mecp2*-heterozygotes.
Figure 9. Cortical and hippocampal discharges are seen in Mecp2-null mouse. A: 10s representative EEG traces from a single recording session of Mecp2-null 08-551 are shown. SWD events are seen during slow wave sleep in the hippocampus, and superimposed on slow delta waves in the somatosensory cortex (top trace) and during awake-immobility as well (lower trace).
Contrary to expectations, *Mecp2*-deficient mice did not show convulsive epileptic discharges. Behavioural convulsions seen, such as tail dorsiflexion or myoclonic jerks, were not accompanied by EEG abnormalities in *Mecp2*-deficient mice. The only epileptiform abnormalities detected were brief, infrequent SWD events. These discharges did not appear to worsen with age and there were no obvious changes in the amplitude, duration, or incidence over time. The other chief EEG abnormality seen was a slight decrease in hippocampal theta frequency in *Mecp2*-deficient mice. Again, there did not appear to be any age-related worsening of the hippocampal theta rhythm. The results offered partial support for the above hypothesis. Epileptiform abnormalities were present; however, convulsive seizures were not seen and EEG abnormalities were not progressive.

4.3. The sharp wave frequency during slow wave sleep does not differ between *Mecp2*-deficient mice and control groups.

Hippocampal traces taken from slow wave sleep segments were used to calculate the incidence of sharp waves in *Mecp2*-heterozygotes and their wild-type counterparts. Although sharp waves were distinctly recognizable in a couple of male *Mecp2*-null subjects, there was insufficient data to calculate a group average. Hence, quantitative sharp wave analysis was not carried out on this group. Visually, sharp waves seen in *Mecp2*-deficient
mice showed a similar profile to wild-types, with a sharp, narrow waveform (figure 10a, 11a). Sharp waves occurred sporadically; consequently, inter-sharp wave intervals varied considerably within the same subject. Individual inter-sharp wave interval averages were averaged between mice of the same group to calculate a group mean for Mecp2-heterozygotes (n=4) and their controls (n=3). Standard deviations of the mean were high for both groups, SD=0.83 for Mecp2-heterozygotes and 0.22 for controls. There was no significant difference between the average inter-sharp wave interval of mutant and control groups as illustrated in figure 11e (1.82 vs. 1.22Hz, p = 0.29). The results therefore went against the initial hypothesis that predicted more frequent sharp waves in Mecp2-deficient mice.
Figure 10. Network activity is preserved in female *MeCP2*-heterozygous mice during sleep. **A, B:** Representative EEG traces of CA1 hippocampus (hipp) and somatosensory cortex (cort) in *MeCP2*-heterozygous (A) and wild-type mice (B) during slow wave sleep. Data segments in gray boxes expanded below. Arrows indicate sharp wave events. **C, D:** Histograms depicting the average peak frequency of oscillations in the hippocampus (C) and cortex (D) during slow wave sleep (mean ± SE). **E:** Histogram depicting average time intervals between sharp wave events (mean ± SE).
Figure 11. Network activity is preserved in male MeCP2-null mice during sleep. A, B: Representative EEG traces of CA1 hippocampus (hipp) and somatosensory cortex (cort) in MeCP2-null (A) and wild-type mice (B) during slow wave sleep. Data segments in gray boxes expanded below. Arrows indicate sharp wave events. C: Histogram depicting the average peak frequency of oscillations in cortex during slow wave sleep (mean ± SE).
4.4. Slow cortical oscillations during slow wave sleep appear normal in *Mecp2*-deficient mice groups.

*Mecp2*-deficient mice showed normal hippocampal and cortical activity during sleep. During slow wave sleep, cortical delta oscillations were detected in both *Mecp2*-deficient mice and control groups (figure 10a, 10b, 11a, 11b). Power spectrum analysis was used to calculate the peak delta frequency of each slow wave sleep segment, and peak frequencies were averaged to produce a mean peak frequency for each animal. Individual averages were then averaged across mice from the same group to calculate mean peak delta frequency for *Mecp2*-heterozygotes (n=6), their controls (n=3), *Mecp2*-null mice (n=3) and their controls (n=3). The peak frequency of cortical delta oscillations did not differ between *Mecp2*-heterozygotes and their controls (2.1 vs. 2.1Hz, p=0.5, figure 11d). In male mutants, delta frequency was slightly slower than in their controls, 1.9 vs. 2.4Hz, but this difference was not found to be significant (p=0.24, figure 11c). Inter-subject variation in peak delta frequency was large, reflected in the high standard deviation values for *Mecp2*-heterozygotes (SD=0.53), their controls (SD=0.38), *Mecp2*-null mice (SD=0.58) and their controls (SD=0.51). These results suggest that network activity is preserved in the sleep behavioural state and support the initial hypothesis.
5. DISCUSSION

5.1. Re-examination of Objective and Hypotheses

The objective of this thesis was two-fold: (1) to describe and compare basal hippocampal and cortical EEG activity in symptomatic-aged \textit{Mecp2}-deficient mice and their controls, and (2) in the event of EEG abnormalities and/or seizures, determine whether they are amenable to pharmacological treatment. The main hypothesis of this thesis was that insufficient levels of MeCP2 in the mouse brain would disrupt normal network activity, resulting in epileptiform EEG patterns in the hippocampus and cortex, and that this phenotype would be more severe in the \textit{Mecp2}-null group.

Collectively, the results obtained in this study do not support the main hypothesis. While subtle alterations to the hippocampal theta rhythm were shown to occur and absence-like events were observed in several mutants, brain network activity was largely preserved and convulsive seizures were not detected in any of the \textit{Mecp2}-deficient mice.

Hypothesis (1)

\textbf{Hippocampal theta network oscillations will show impairments in frequency and amplitude.} Exploratory theta segments will be shorter in \textit{Mecp2}-deficient mice.
Previous studies have found severe impairments in hippocampal-dependent learning in MeCP2-deficient mice (Moretti et al, 2006; Stearns et al, 2007). Given the direct link between hippocampal theta and spatial learning (O’Keefe and Reece, 1993), one would predict a significant impairment to the hippocampal theta rhythm of MeCP2-deficient mice. Specifically, a reduction in the frequency and amplitude of EEG theta oscillations would be expected. The results of this study partially support the above hypothesis; however, alterations to the hippocampal theta rhythm were subtle. EEG theta oscillations displayed a relatively normal profile (RESULTS 4.1) and were stable over the course of exploratory behaviours.

The average length of exploratory theta episodes did not differ between MeCP2-deficient mice and wild-types. On the surface, this result appears to contradict findings that MeCP2-deficient mice are hypoactive (Shahbazian et al, 2002; Guy et al, 2001; Chen et al, 2001); however, this is not necessarily the case. MeCP2-deficient mice may explore less frequently than their wild-type counterparts, but when they do explore, it is for similar durations.

Theta amplitude was not compared among MeCP2-deficient mice and control subjects as the unnormalized amplitude varied considerably between subjects of the same group and within the same animal. One common reason for inter-individual variations in theta amplitude is the depth of the recording electrode. The phase and amplitude of theta
oscillations vary according to depth in the hippocampus. Beginning in the cortex immediately above to the hippocampus, and moving down through the hippocampal layers, the amplitude at first gradually increases with electrode depth until a maximum is reached in the pyramidal cell layer. Just below the pyramidal cell layer, a minimum is reached and theta phase is gradually shifted until full (180 degree) reversal is reached in the stratum lacunosum moleculare. Theta amplitude gradually increases as the recording electrode is moved from the stratum radiatum downwards, and is largest in the hippocampal fissure (Bragin et al, 1995). While recording electrode lengths were consistent in all subjects of this study, individual variations in brain anatomy could result in electrodes reaching different depths.

Amplitude variations may occur within the same animal due to changes in the resistance of the recording apparatus. In this study, the general trend was that hippocampal amplitude decreased over time, an effect which may in part be explained by the accumulation of brain matter around the recording electrode site.

The frequency of hippocampal theta oscillations was conclusively lower in *Mecp2*-deficient mice. Hippocampal theta frequency was decreased ~10% in *Mecp2*-heterozygous mice and ~20% in *Mecp2*-null mice compared to controls. While highly significant, the impairment in hippocampal theta frequency was not nearly as drastic as predicted. *In vitro* studies have found a decrease of approximately 50% in the frequency of spontaneous,
intrinsic hippocampal oscillations (Zhang et al, 2008), and an effect of similar magnitude had been expected. The decrease in the frequency of hippocampal theta oscillations may in part account for the impairments in spatial dependent learning. Deficits in synaptic plasticity observed in MeCP2-deficient mice (Asaka et al, 2006; Moretti et al, 2006) could also explain impairments in hippocampal-dependent learning.

An alteration of the inputs driving hippocampal theta could account for the decreased theta frequency seen in MeCP2-deficient mice. As discussed in INTRODUCTION 1.4.1 the medial septum sends cholinergic and GABAergic inputs to the hippocampus, while the entorhinal cortex sends glutamatergic inputs to regulate hippocampal theta rhythm.

**Cholingeric input**

Blockade of cholinergic input to the hippocampus is known to reduce the amplitude of theta oscillations, but has minimal effect on frequency (Lee et al, 1994). Therefore, reduced cholinergic input could not account for the observed impairments to the hippocampal theta rhythm.

**GABAergic input**

Increased GABAergic input to the hippocampus, via the medial septum, would be expected to decrease the frequency of hippocampal theta oscillations. Anxiolytic drugs which act to
increase GABA\textsubscript{A} transmission have been found to decrease the frequency of reticular-elicited hippocampal theta in a dose-dependent manner (McNaughton, 2007). However, there have been no reports of excessive GABAergic input to the hippocampus.

**Glutamatergic input**

A reduction in the glutamatergic input from the entorhinal cortex would also be expected to decrease the frequency of hippocampal theta (Buzsaki, 2002). Both pre- and post-synaptic mechanisms might decrease the regulatory glutamatergic drive to the hippocampus. One *in vitro* study reported decreased excitatory drive onto layer 5 pyramidal neurons of *Mecp2*-null mice (Dani et al, 2005). Presumably, a decrease in cortical input to the entorhinal cortex via layer 5 pyramidal neurons would in turn decrease glutamatergic output to the hippocampus. Belichenko et al (2009) found that dendritic spine density was decreased \(\sim 11\%\) in the dentate gyrus, and nearly 20\% in the CA1 hippocampus of *Mecp2*-null mice. Since dendritic spines are the principal sites of excitatory neurotransmission, such a decrease could post-synaptically reduce the response to glutamatergic input.

**Intrinsic oscillatory activity**

A complementary explanation for the reduced hippocampal theta frequency may be that the intrinsic oscillatory activity of the hippocampus is decreased in *Mecp2*-deficient mice. In support of this explanation, Zhang et al (2008) found a decrease of \(\sim 50\%\) in the frequency of spontaneously occurring network oscillations in the CA3 hippocampus of *Mecp2*-null
mice, which they concluded was in part due to decreased basal glutamatergic drive, either through pre- or post-synaptic mechanisms.

**Hypothesis (2)**

Epileptic seizures will occur in *Mecp2*-deficient mice. Specifically, generalized convulsive and myoclonic seizures will be seen. EEG abnormalities will show a progression similar to the stages in Rett girls.

The results of the current study partially supported the above hypothesis. Epileptiform-like cortical slow spike and wave discharge (SWD) events did occur in the majority of 9-14 month old Mecp2-heterozygous mice. These discharges were likely absence seizures as they showed a similar profile to SWD events seen in absence rodent models, with the following similarities to the GAERS model:

1. SWD events occurred during behavioural immobility, or when moving, in which case movement was suddenly interrupted and then resumed as soon as the cortical discharge stopped (Danober et al, 1998).

2. They were only seen in the somatosensory cortex channel, and did not occur in the occipital cortex where the ground electrode was positioned (or SWD events would have appeared in the hippocampal channel as well). This is consistent with the
GAERS model, where SWDs were predominantly seen in the fronto-parietal region, and rarely in the occipital cortex (Danober et al, 1998).

(3) SWDs showed a similar waveform to those seen in rodent models of absence epilepsy.

(4) Discharge onset was sudden and there was no post-ictal depression.

(5) Lastly, SWDs responded to treatment with ethosuximide, a T-type Ca2+ channel blocker which is routinely used in humans with absence epilepsy. Only one mouse, J2 failed to respond to ethosuximide treatment, possibly because a higher dosage was required. Other studies on absence mice models found ethosuximide to be effective at doses higher than the 100mg/kg (Hosford et al, 1992; Heller et al, 2007).

The genetic background of MeCP2-deficient mice was not likely a contributing factor to the generation of SWDs. While spontaneous SWDs are known to occur in inbred strains of mice, C57BL/6 is one among the least susceptible to seizures (Tan et al, 2008). Further, rhythmic SWD events were not seen in any of the wild-type mice recorded.

Surprisingly, in the more severe MeCP2-null group, only one of the four mutants recorded from showed absence-like discharges. However, the discharges seen in 08-551 showed a different profile to those in MeCP2-heterozygotes, occurring in both hippocampal and somatosensory cortex channels. They also occurred during slow wave sleep,
superimposed on slow cortical delta oscillations. Thus, the rhythmic discharges seen in

*Mecp2*-null mice may represent an atypical form of absence epilepsy.
Figure 12. (Modified from Beenakker and Huguenard, 2009). Sleep spindles and spike and wave discharges during absence seizures relies on thalamocortical oscillations between inhibitory neurons of the reticular thalamic nuclei (RT) and excitatory thalamocortical neurons (TC) as shown below. RT neurons paradoxically activate TC neurons through deinactivation of voltage-sensitive Ca2+ channels. Rhythmic oscillations are spread to the cortex via TC projections to the cortex. Shown above, the amplitude of thalamocortical oscillations depends on both intra-RT inhibition, which limits the number of RT neurons contributing to these oscillations, and on gap junctions between RT neurons, which synchronizes thalamocortical oscillations.
The above findings point to a misregulation in the thalamocortical circuitry of MeCP2-deficient mice. The thalamocortical circuitry is believed to be responsible for the generation of spike and wave discharges associated with absence seizures. Spike and wave discharges are believed to “hijack” the circuitry involved in sleep spindle generation (Beenhakker and Huguenard, 2009). In sleep spindle generation, ~10Hz oscillations are generated by the interplay of inhibitory neurons of the reticular thalamic nuclei (RT) and excitatory thalamocortical neurons (TC), and rhythmic activity is spread to the cortex (Huguenard and McCormick, 2007). As shown in figure 12, RT neurons send inhibitory GABAergic projections to TC neurons, activating fast, GABA\textsubscript{A} and slow, metabotropic GABA\textsubscript{B} receptors. TC neurons project to the cortex and send collaterals to RT neurons (Beenhakker and Huguenard, 2009). Both RT and TC neurons receive excitatory input from the cortex; however, the activation of RT neurons is stronger and the net effect is thus to inhibit TC neurons. RT neurons cause bursting in TC neurons through paradoxical activation. Inhibitory input to TC neurons deinactives low-threshold T-type Ca\textsuperscript{2+} channels, producing Ca\textsuperscript{2+} spikes which in turn trigger bursts of action potentials. The alternation between RT and TC neuronal bursting produces oscillations around ~10Hz. The frequency of these oscillations depends on the relative contribution of fast GABA\textsubscript{A} and slow GABA\textsubscript{B} receptor activation on TC neurons. The amplitude of these oscillations depends on the degree of intra-RT inhibition. RT neurons inhibit each other to prevent hypersynchronous responses from occurring during sleep spindle generation (Beenhakker
Intra-RT inhibition relies on GABA\textsubscript{A} receptors. When GABA\textsubscript{A} receptor blockers are applied to thalamic slices, sparse spindle activity is transformed into slower, robust oscillations. Similarly, when GABA\textsubscript{A} receptor blockers are locally injected into the thalamus of behaving animals, robust spike and wave discharges are observed (Castro-Alamancos, 1999). Presumably, blockade of GABA\textsubscript{A} receptors transforms low amplitude, spindle-like oscillations into robust spike and wave discharges through blockade of intra-RT inhibition, which allows RT neurons to synchronously discharge. Spike and wave discharges associated with absence seizures may be of a slower frequency than spindle oscillations because the slower GABA\textsubscript{B} component contributes relatively more to the hyperpolarizing waveform in TC cells (Beenhakker and Huguenard, 2009). Thus, one possible explanation for the presence of absence like SWDs in \textit{Mecp2}-deficient mice is that GABA\textsubscript{A} transmission is impaired. In support, Zhang et al (2008) found that low doses of GABA\textsubscript{A} receptor antagonist bicuculine were able to produce spike-like waveforms in \textit{Mecp2}-null but not wild-type hippocampal slices in vitro, suggesting the GABA\textsubscript{A} system may be misregulated. However, there is no evidence to suggest a GABA\textsubscript{A} misregulation in the thalamocortical circuitry. Alternatively, basal GABA\textsubscript{B} transmission may be increased in \textit{Mecp2}-deficient mice.

In this study, clinical convulsive seizures were not seen in \textit{Mecp2}-deficient mice. By contrast, generalized convulsive seizures are very common in Rett girls whereas typical absence seizures are rare (Glaze, 2005). This finding is at odds with a prior EEG
investigation on the Mecp2<sup>308/Y</sup> model in which several mice showed myoclonic seizures. However, it is important to note that the Mecp2<sup>308/Y</sup> mice were maintained on a different background, which may be more prone to developing epileptic discharges, and were subjected to a more stressful implantation procedure (screw-based) which could also have influenced seizure outcome.

Despite the large variability in EEG recordings of Rett girls, common trends emerge including slowing of baseline activity in the awake state, loss of the occipital dominant (alpha) rhythm, and epileptiform-like activity appearing first during sleep and in later stages during wakefulness (Hagberg et al, 1983). One limitation of this study was that Mecp2-deficient mice were not recorded at younger ages. Consequently, the EEG progression from the asymptomatic stage to the symptomatic stage could not be studied. The cortical EEG of Mecp2-deficient mice was expected to show a reduced high frequency component in the immobile-aware state, in line with the marked slowing of background activity reported in Rett girls (Hagberg et al, 1983). However, cortical and hippocampal EEG traces displayed a normal frequency distribution in all behavioural states. Power spectrum plots showed a clear peak in the theta range during exploration, cortical delta oscillations during sleep, and a broad distribution over the 1-5HZ range during immobility. EEG abnormalities were expected to become more pronounced with age, based on findings that the EEG of Rett girls follows a staged progression (Glaze, 2005). However, the reduction in hippocampal theta frequency did not change over time in the few Mecp2-
heterozygous mice recorded beyond the age of 14 months (see RESULTS 4.1). Also, the profile and incidence of SWD events did not appear to change with age. In summary then, EEG abnormalities were not progressive in 9 month old plus Mecp2-deficient mice.

**Hypothesis (3)**

**Sharp wave discharges during immobility will be more frequent.**

As discussed in INTRODUCTION 1.4.1 sharp waves reflect the synchronous firing of thousands of hippocampal pyramidal cells. It was hypothesized that Mecp2-deficient mice would have a greater propensity for sharp wave generation based on clinical findings from Rett girls and mice that the Rett brain is prone to hypersynchrony (Glaze, 2005; Zhang, 2008). The results do not support the above hypothesis and counter the view that the Mecp2-deficient brain is epileptogenic. The inter-sharp wave interval was not significantly different between Mecp2-heterozygotes and wild-type mice. However, the results must be interpreted with caution. Sharp wave events were not quantified in the more severe, Mecp2-null group, due to insufficient numbers. Also, statistical issues such as large within-group variation and small sample size might have prevented a true difference in sharp wave frequency from being detected in Mecp2-heterozygous mice. Another argument is that Mecp2-deficient mice may need to be challenged to expose their underlying epileptogenic phenotype. Hippocampal sharp wave events did not occur spontaneously in
Mecp2-null slices, but did so when a low dose of the GABA\(_A\) receptor blocker, bicuculine, was applied, or in response to high frequency stimulation of the CA3 hippocampus (Zhang et al, 2008).

**Hypothesis (4)**

**Delta oscillations during slow wave sleep will have a normal frequency distribution in Mecp2-deficient mice.**

Cortical delta oscillations were expected to show a normal frequency distribution in Mecp2-deficient mice. Although EEG disturbances are common during sleep in Rett girls, there have been no reports of changes to the profile of slow, delta oscillations. Moreover, slow sleep oscillations are based largely on channel properties (INTRODUCTION 1.4.1) which appear to be preserved in the Rett brain. The above hypothesis is supported by the results of this study. The average peak delta frequency during slow wave sleep was nearly identical between Mecp2-heterozygotes and wild-types, despite large variation within the Mecp2-heterozygous group. Cortical delta oscillations had a slightly lower peak in the Mecp2-null group relative to controls, but this difference was not found to be statistically significant. These findings support an emerging theme of this thesis which is that the network activity of Mecp2-deficient mice is largely preserved.
5.2. Relation of Findings to Literature

The study of Rett Syndrome has advanced greatly since the discovery of the causal gene mutation, *MECP2* (Amir et al, 1999). MeCP2 is highly expressed in mature neurons and its loss has a primarily neurological effect. Several *Mecp2*-deficient mice models have been established and have been used to study the effect of insufficient MeCP2 in terms of brain morphology (Belichenko et al, 2009), neurochemistry (Jellinger, 2003), synaptic plasticity (Asaki et al, 2006; Moretti et al, 2006) and network activity (Zhang et al, 2008). What is lacking in this area of research is an understanding of how *in vitro* findings extend to *in vivo* changes in network activity and seizures. Epilepsy remains a prominent part of Rett Syndrome and is not always well controlled. The problem with EEG studies on Rett girls is that there is such huge variability (see INTRODUCTION 1.1.4) and it is therefore difficult to make generalized statements. With the exception of one study (Shahbazian et al, 2002) all electrophysiological studies of Rett mice have been conducted using brain slices. *In vitro* studies are useful as a starting point, but are somewhat limited as they can not assess brain function with all connections intact. The single *in vivo* study on Rett mice was shallow in depth, and did not look at physiological patterns of network activity in the brain such as the hippocampal theta rhythm, or cortical delta oscillations (Shahbazian et al, 2002).

The results of this thesis conflict clinical observations from Rett girls and *in vitro* studies of *Mecp2*-deficient mice. With respect to baseline EEG activity, the results of this
study have shown surprisingly little difference between *Mecp2*-deficient mice and wild-types. *Mecp2*-null hippocampal slices showed a ~50% reduction in the frequency of intrinsic oscillations (Zhang et al, 2008) while hippocampal theta frequency was only subtly impaired *in vivo*. The only seizure type observed in this current study was a transient, absence-like spike and wave discharge which occurred in a subset of the *Mecp2*-deficient mice. By contrast, multiple seizure-types are often present in Rett girls and generalized convulsive seizures are most common (Glaze, 2005). *In vitro* hippocampal studies have shown that the *Mecp2*-deficient brain is prone to hyperexcibility and readily develops sharp wave-like discharges (Zhang et al, 2008). However, the inter-sharp wave interval calculated during slow wave sleep did not differ between *Mecp2*-heterozygotes and wild-types. Finally, EEG abnormalities were not progressive in 9-14 month old *Mecp2*-deficient mice whereas the EEG of Rett girls follows a staged progression (Hagberg et al, 1983).

Although previous *in vitro* findings from Rett mice did not correspond with the *in vivo* changes in network activity reported here, this does not invalidate any of these studies. *In vitro* studies are useful for understanding the intrinsic activity of a particular circuit, as input from other brain regions is severed. Studies on hippocampal slices best resemble the sleep behavioural state during which all cortical input is removed. *In vivo* recordings on the other hand, consider global network patterns, with all connections intact. The results of this study may not agree with findings from Rett girls for reasons that will be considered in greater detail in DISCUSSION 5.3.
5.3. Re-evaluating the Rett Mouse Model

Despite the usefulness of non-primate models of human disease, there are often cases in which animal models do not correspond to their human counterparts. To give an example, mice lacking a copy of the CFTR gene, responsible for the cystic fibrosis condition in humans, do not exhibit classic features of cystic fibrosis. Rather than suffering from pulmonary obstruction, these mice have severe gastrointestinal obstructions due to species-specific differences in tissue expression patterns of the CFTR gene (Davidson and Rolfe, 2001).

There is good reason to challenge the validity of MeCP2-deficient mice models of Rett Syndrome. As previously discussed, MeCP2-deficient mice exhibit more subtle abnormalities than Rett patients. Mice heterozygous for the MeCP2 gene display mild behavioural and EEG impairments, and live a normal lifespan. Male MeCP2-null mice live into adulthood whereas boys with MECP2 mutations die in infancy (Schule et al, 2008). The transcriptional regulator MeCP2 may thus play a more prominent role in the human brain. Alternatively, genetic modifiers such as BDNF polymorphisms may produce species specific differences in the progression of Rett Syndrome, which may in part explain the absence of convulsive seizures and milder Rett phenotype in mice. BDNF expression has been shown to influence epileptiform activity in wild-type rodents. Increased BDNF levels
lead to cortical hyperexcitability and epileptiform activities while suppression of BDNF was found to retard the development of seizures in rodents (Xu et al, 2004).

The neurotrophic factor BDNF may be an important regulator of MeCP2. When MeCP2-null mice were crossed with mice lacking BDNF to produce a double knockout, Rett-like symptoms appeared earlier in the resulting mutants. Locomotory deficits appeared at 4 rather than 6 weeks, and lifespan was decreased. The opposite effect was observed in MeCP2-null mice overexpressing BDNF. Disease onset was delayed and life expectancy prolonged (Chang et al, 2006). Furthermore, enhancement of BDNF levels in a mouse model of RTT lead to improvements in the Rett phenotype, ameliorating respiratory symptoms (Ogier et al, 2007). As discussed in INTRODUCTION 1.1.4.3, the severity of RTT may depend on which BDNF polymorphism is present. The V66M BDNF allelic variant is associated with earlier seizure onset and slightly increased severity, and occurs in roughly a quarter of Rett girls (Egan et al, 2003). This BDNF allele is not found in rodents which may in part explain the mild phenotype observed in MeCP2-deficient mice models.

Baseline differences in EEG network patterns often exist between species. Hippocampal theta-range oscillations are prominent in non-primate mammals during exploration yet there is a debate as to whether humans have an equivalent hippocampal theta rhythm. The frequency of absence spike and wave discharges is 3Hz in humans but in the theta range (6-10Hz) in rodent models of absence epilepsy (Danober et al, 1998). EEG
findings from the human brain may not extend to the mouse brain and vice versa due to anatomical differences in brain structure and/or circuitry. The brain regions recorded from in this study, the hippocampus and somatosensory cortex, show important species-specific differences (see below) which may in part explain the discrepancy between the EEG of Rett girls and the findings of this thesis.

Basic connectivity patterns are consistent between the mouse and human hippocampus; however, important differences exist in their circuitry and structure. The rodent hippocampus has heavy commissural (inter-hemispheric) projections in the dentate gyrus and CA3 regions whereas the human hippocampus is nearly devoid of commissural projections (O'Keefe, 2006). In rodents, the CA1 pyramidal cell layer is 5 cells thick. By comparison, this layer is more than 30 cells thick in the human hippocampus. The entorhinal cortex, the main source of cortical innervations to the hippocampus via the perforant pathway, is greatly expanded and differentiated in the human brain as a result of stronger interconnections with associational (polysensory) areas of the neocortex. In rodents there are two distinct subdivisions of the entorhinal cortex versus at least eight distinct subdivisions in the human brain. Hippocampal functioning is more dependent and integrated with the neocortex in the human brain (O'Keefe, 2006). Both rodents and humans have a six-layered neocortex; however, in humans there is a huge expansion in the number of neurons in superficial cortical layers.
5.4. Novelty and Significance

The behavioural phenotypes of Rett mice models have been well established and several studies have examined the electrophysiology of Mecp2-deficient mice in vitro. In vitro recordings reflect the intrinsic activity of a particular brain region; recordings of hippocampal slices closest resemble electrical activity in the sleep state, in which all cortical input is removed. In vivo recordings are necessary for understanding brain network activity, under physiological conditions, with all connections intact. To date, only one study has investigated the Mecp2-deficient brain in vivo.

Several important differences exist between this current study and the EEG study outlined in Shahbazian et al (2002). First, different Rett mice models were investigated. While the Zoghbi group used the male Mecp2^{308Y} model, this study used the Bird Mecp2-null and Mecp2-heterozygous mice models. Secondly, different brain regions were studied. While the Zoghbi study was restricted to the cortex, both the hippocampus and somatosensory cortex were examined here. Lastly, the Zoghbi study was shallow in depth and only reported epileptiform activity. A more thorough characterization of abnormal epileptiform activity as well as physiological network activity was conducted here. This current study thus presents the first true EEG assessment of Mecp2-deficient mice.
Another novelty of this study was the implantation of intracranial electrode caps, super-glued to the skull. Intracranial depth electrodes allowed deep brain structures, such as the CA1 hippocampus, to be studied. In contrast, subdural electrodes (used in Shahbazian et al, 2002) record only surface EEG activity, and offer poorer spatial resolution. The use of super-glue greatly increased the stability of electrode caps over traditional screw-based methods. Stable recordings were produced over 6 months after the date of implantation, allowing age-dependent EEG trends to be studied in MeCP2-deficient mice.

The EEG of MeCP2-deficient mice showed surprising little similarity to the severely abnormal EEG of Rett girls; basal network activity was only subtly impaired. Together with behavioural observations that the phenotype of Rett mice is far milder than their human counterparts, these results suggest that the function of MeCP2 may vary across species. Thus, findings from Rett mice may not extrapolate to Rett patients. This is one of the first studies to openly challenge the validity of mice models of Rett Syndrome.

5.5. Potential Pitfalls and Limitations

(1) Small sample size

A more complete EEG characterization of MeCP2-deficient mice was hindered by several factors. Most importantly, sample size was limited, especially in the case of MeCP2-null mice. This was in part due to the limited availability of these mice. In addition, early
implantation procedures (using Avertin) had a high fatality rate for Mecp2-null mice and less so for Mecp2-heterozygotes. The administration of isoflurane inhalational anesthetic during implantation improved the success rate to nearly 100% in mutants. Inter sharp wave intervals could not be accurately compared between Mecp2-heterozygous and wild-type due to large variation, and could not be statistically calculated in the Mecp2-null group due to insufficient numbers.

(2) Lack of perturbation

Convulsive seizures did not occur in the naïve, unperturbed state in Mecp2-deficient mice recorded in this study. Myoclonic seizures were reported in the Shahbazian et al (2002) study, however, there mice were subjected to a more stressful electrode implantation procedure and maintained on a background more prone to developing seizures. It is possible that Mecp2-deficient mice have increased seizure susceptibility, and develop seizures more readily than wild-type mice, but must be provoked in order to expose their underlying epileptogenic phenotype. In support of this view, sharp wave-like events occurred in vitro in response to a low dose of bicuculine, but were not seen under basal conditions in hippocampal slices (Zhang et al, 2008). Various challenges such as hypoxia, kindling, or pharmacological manipulations, could have tested this hypothesis.

(3) Applicability to humans
Animal models may be extremely useful for studying human conditions, especially when important questions can not be answered in human subjects due to ethical considerations or invasiveness. However, species differences often arise limiting the usefulness of animal models. *Mecp2*-deficient mice display a much milder phenotype than their human counterparts. *Mecp2*-null mice have widely been accepted as a model for Rett Syndrome, yet they completely lack MeCP2, which tells us that MeCP2 plays a unique, more prominent role in humans and there are clear species differences in functionality.

**4) Electroencephalographic seizures**

The lack of classic convulsive electroencephalographic seizures may be due to a variety of reason. It is possible the movement artifacts masked EEG signals during seizure-like behaviours such as tail-smacking, and myoclonic jerks. However, there were no high frequency oscillations detected during these events suggesting that there was no EEG correlate to seizure-like behaviours in the areas recorded from. Another possibility is that seizures did occur, but very infrequently and were therefore not detected. 24 hour recordings could have addressed this possibility.

**5) Experimental Parameters**

Due to software filtering of the EEG, slower delta oscillations (those in the 0.5 to 1 Hz range) could not be detected. Therefore, the frequency distribution during slow wave sleep may not accurately reflect this component of delta sleep. Only oscillations above 0.667 Hz
were detected, and with the 0.41Hz resolution used these oscillations would appear at 0.82 Hz. The relatively thick electrode size (150um) prevented higher frequency “ripple” oscillations from being detected. High frequency oscillations in the ripple range occur in pathological conditions, such as medial temporal lobe epilepsy, and may have been present in the Rett brain. Another major limitation of the study was that amplitude differences could not be compared due to normalization issues. There was no consistent reference signal to which hippocampal and cortical EEG signals could be normalized. EEG signals could not be normalized to higher, non-physiological signals because these signals represented noise, and varied considerably between recording sessions.

5.6. Conclusions

Two main abnormalities were seen in Mecp2-deficient mice. The frequency of hippocampal EEG theta oscillations was significantly reduced, especially in the Mecp2-null group. This outcome may be explained by the reported decrease in the intrinsic oscillatory frequency of the hippocampus, postulated to be result of reduced basal glutamatergic drive, together with reduced excitatory glutamatergic input from the entorhinal cortex. The entorhinal cortex may project less heavily to the dentate gyrus and CA1 regions as a result of decreased cortical input from layer 5 neurons. Post-synaptically, hippocampal neurons
may be less responsive to glutamatergic input due to a decrease in the spine density of these neurons.

The other main abnormality seen was the presence of spontaneous spike and wave discharges. These discharges are likely absence seizures and may result from a disruption to the thalamocortical circuitry in the MeCP2-deficient mouse brain. All other aspects of brain network activity appeared normal in the mutant mice. In summary then, the Rett mouse model poorly correlated with Rett girls from an electrophysiological perspective, and may be an inappropriate model for studying the EEG of Rett patients.

5.7. Future Directions

The current approach to the clinical management of RTT is highly symptom-based. Problems commonly associated with Rett Syndrome, such as ambulatory difficulties, gastrointestinal disturbances, breathing irregularities, communication difficulties and epilepsy, are separately addressed. Consequently, Rett girls are often heavily medicated and on multiple treatment programs. Despite recent advances in our understanding of RTT, the prognosis still remains grim; the majority of Rett girls are unable to live independently their entire lives. A more global treatment approach, which addresses the root cause of Rett Syndrome, is needed.
Studies on MeCP2-deficient mice models have demonstrated the proof of principle that Rett Syndrome is reversible. When MeCP2 was introduced to MeCP2-null males, functional recovery was observed, even at advanced stages of RTT where hindlimb clasping, tremor and irregular breathing were present. Full length MeCP2 was detected and mice lived well beyond the expected 17 weeks (Guy et al, 2007). Unfortunately, MECP2 introduction is not a practical strategy for the treatment of Rett girls. The expression of MECP2 must be tightly regulated. Overexpression, at twice wild-type levels, results in excessive synaptic plasticity, seizures, hypoactivity, and early death (Collins et al, 2004). Introducing an additional copy of MECP2 in Rett girls would result in approximately 50% of cells (depending on X-chromosome inactivation patterns) expressing MECP2 at twice the normal level, with potentially deleterious effects.

Recent studies suggest that a subset of Rett girls may benefit from aminoglycoside or PTC124 treatment (Brendel et al, 2009). Aminoglycosides are a class of antibiotics that contain a sugar and an amino group, and include amikacin, gentamicin, neomycin, and streptomycin (http://en.wikipedia.org/wiki/). Aminoglycosides were serendipitously discovered to induce read-through of premature stop codons (UGA, UAG, UAA) through a mechanism unrelated to its antibiotic effects. At high concentrations, aminoglycosides reversibly bind to ribosomal complexes and interfere with proofreading mechanisms, allowing random amino acids to be integrated to the emerging protein at the position of the premature stop codons (Manuvakhova et al, 2000). Aminoglycosides do not appear to
interfere with normal transcription. The efficiency of aminoglycosides at allowing read-through is highly context-dependent, depending largely on the stop codon present (UGA>UAG>UAA) and the nucleotide immediately after the termination codon (U or A>C or G) (Manuvakhova et al, 2000).

Aminoglycosides have been able to produce partial phenotypic rescue in genetic disorders resulting from nonsense mutations. Gentamicin is currently being tested in patients with cystic fibrosis, a disease caused by mutations to the CFTR gene. One pilot study found that application of gentamicin to the nasal epithelium restored the CFTR chloride channel protein in 90% of subjects with a functional improvement in electrophysiology in 21% and restored functioning of chloride and sodium channels in 68% (Lukacs and Durie, 2003). Gentamicin treatment was less effective at rescuing the mdx mouse model of Duchenne Muscular Dystrophy, a disease caused by mutations to the dystrophin gene. One study found that gentamicin-induced readthrough of the UGA C premature stop codon was only 6% and 1% for the UAA A premature stop codon in vivo (Howard et al, 2000). The aforementioned studies illustrate the context-dependency of aminoglycoside-induced read-through and suggest that genetic conditions will respond differently to treatment.

One of the drawbacks of aminoglycoside treatment is that very high concentrations are required for read-through, producing toxic side effects. Furthermore, even at the
maximal dosage, read-through efficiency may be very low, depending on the context of the nonsense mutation (Welch et al, 2007). Recently, a far more potent compound, PTC124, was discovered through high-throughput screening. PTC124 is structurally unrelated to aminoglycosides. This compound allows read-through of nonsense mutations in a dose-dependent manner, with UGA>UAG>UAA. Maximal read-through of nonsense mutations in the human/ mouse dystrophin gene occurred at a concentration of 5ug/mL. By comparison, maximal read-through occurs at 1mg/mL of gentamicin, a concentration 200x higher. PTC124 read-through efficiency was found to be 40-60% in vitro. In the mdx mouse model, dystrophin protein levels were 20-25% of wild-type levels in muscles when the plasma concentration of PTC124 was kept above 10ug/mL for 2-8 weeks. Partial functional rescue occurred; muscle strength improved and partial protection against injury was noted (Welch et al, 2007). No obvious toxicity was associated with systemic delivery of the drug. PTC124 offers much promise in the treatment of genetic disorders resulting from nonsense mutations and should be tested in other genetic models.

Rett Syndrome is a good candidate for aminoglycoside/ PTC124 treatment as 35.1% of North American cases are caused by nonsense mutations in the MECP2 gene (Percy et al, 2007). Common nonsense mutations include R168X (9.4%), R255X (9%), R270X (7.2%) and R294X (6.2%). In vitro studies have found that gentamicin was moderately successful at rescuing nonsense mutations in the human MECP2 gene in vitro (Brendel et al, 2009). Consistent with other studies, read-through efficiency was found to be context dependent,
largely depending on the stop codon present (UGA>UAG>UAA) and the nucleotide following the premature stop codon (U>A>G). Maximal read-through efficiency occurred at a concentration of 1mg/mL gentamicin. At this concentration, read through efficiency was as follows: R168X 10%, R255X 13.5%, R270X 11.8%, R294X 21.8% (Brendel et al, 2009). Several considerations may limit the usefulness of gentamicin in treating nonsense mutations in Rett Syndrome. First, the concentration required for maximal read-through, 1mg/mL, would be highly toxic to Rett girls. Secondly, RTT is primarily a neurological disorder and functional rescue would therefore require gentamicin to cross the blood brain barrier.

Nonetheless, it would still be beneficial to test whether gentamicin or PTC124 was able to produce functional rescue in vivo. PTC124 would be an ideal candidate for treating nonsense mutations in MeCP2-deficient mouse models. Alternatively, lower doses of gentamicin could be applied over a longer time course. PTC124 or gentamicin should be tested at different concentrations to determine the ideal dosage. PTC124- or gentamicin-treated Rett mice and their controls could be assessed for neurological rescue using established behavioural (e.g. novel object recognition) and electrophysiological parameters. The methods used to assess basal brain activity in this thesis could be applied to determining whether or not neurological rescue occurred, and if this effect was long-term. EEG recordings of the hippocampal and somatosensory cortex regions could be taken.
Normal hippocampal theta frequency and amplitude, and the absence of spike and wave discharges or other epileptiform activity would indicate functional rescue.
6. REFERENCES


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