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# Electrographic and pharmacological characterization of a progressive epilepsy phenotype in female MeCP2-deficient mice

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ABSTRACT

Rett Syndrome is a neurodevelopmental disorder caused primarily by mutations in the gene encoding Methyl-CpG-binding protein 2 (*MECP2*). Spontaneous epileptiform activity is a common co-morbidity present in Rett syndrome, and hyper-excitable neural networks are present in MeCP2-deficient mouse models of Rett syndrome. In this study we conducted a longitudinal assessment of spontaneous cortical electrographic discharges in female MeCP2-deficient mice and defined the pharmacological responsiveness of these discharges to anti-convulsant drugs. Our data show that cortical discharge activity in female MeCP2-deficient mice progressively increases in severity as the mice age, with discharges being more frequent and of longer durations at 19–24 months of age compared to 3 months of age. Semiologically and pharmacologically, this basal discharge activity in female MeCP2-deficient mice displayed electroclinical properties consistent with absence epilepsy. Only rarely were convulsive seizures observed in these mice at any age. Since absence epilepsy is infrequently observed in Rett syndrome patients, these results indicate that the predominant spontaneous electroclinical phenotype of MeCP2-deficient mice we examined does not faithfully recapitulate the most prevalent seizure types observed in affected patients.

## 1. Introduction

Rett syndrome is an X-linked neurodevelopmental disorder caused by mutations in the gene encoding Methyl-CPG-binding protein 2 (*MECP2*) and is one of the leading genetic causes of severe intellectual disability in females (Amir et al., 1999). Rett syndrome is characterized by normal development up to 6–18 months of age followed by a rapid regression of acquired skills and the development of motor impairments, cognitive abnormalities, and autonomic dysfunction (Hagberg et al., 1983; Weese-Mayer et al., 2006; Kaufmann et al., 1992). One particularly severe co-morbidity affecting 60–80% of Rett syndrome patients is epileptic seizures, which tend to be poorly controlled by classical anti-convulsant medications (Huppke et al., 2007; Nissenkorn et al., 2010; Vignoli et al., 2017). In most cases, the seizure onset occurs around 2 years of age, and the severity of the seizures may increase until 7–12 years of age (Cooper et al., 1998; Glaze et al., 2010; Jian et al., 2007). There appears to be a variety of seizure types that can affect Rett syndrome patients, and in many instances different seizure types can co-exist within the same patient. Further electroencephalographic (EEG) abnormalities such as generalized spike-wave discharges also may be observed in Rett patients at different stages of disease progression (Glaze, 2005; Krajnc, 2015). Prevalent types of seizures observed in Rett syndrome patients include complex partial, tonic/atonic (Niedermeyer et al., 1986; Huppke et al., 2007), with typical or atypical absence being only infrequently seen (Pardal-Fernández et al., 2004; Krajnc, 2015). To date, however, the mechanisms through which epileptiform activity arises in Rett syndrome remain poorly understood.

Several mouse models of Rett syndrome have been developed that either lack Mecp2, or express a clinically-relevant mutant form of Mecp2 (Katz et al., 2012). These transgenic mice recapitulate several of

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the cardinal behavioral and neurological deficits observed clinically in Rett syndrome patients expressing different mutations of *MECP2*. One such phenotype caused by the absence of MeCP2 is neural network hyper-excitability (Zhang et al., 2008; Calfa et al., 2011; Ward et al., 2011; Taneja et al., 2009; McLeod et al., 2013). Spontaneously occurring cortical spike and wave EEG discharges have been demonstrated in freely moving male and female mice expressing a null *Mecp2* allele (D'Cruz et al., 2010; Wither et al., 2012; Lang et al., 2014), or a clinically-relevant mutant form of *Mecp2* (Shahbazian et al., 2002; Goffin et al., 2011; Goffin et al., 2014). As with patients however, the nature of epileptiform discharge activity in these models remains only minimally investigated. Therefore, the aims of our present study are to examine cortical epileptiform discharge events in MeCP2$^{+/-}$ female mice at different stages of life, and define their pharmacological responsiveness to anti-convulsant drugs.

## 2. Materials and methods

### 2.1. Ethics statement

Experimentation on animal subjects was conducted in accordance with Canadian Council of Animal Care guidelines, with animal use protocols reviewed and approved by local animal care committees prior to experimental onset (UHN Animal Use Protocol 1321.10).

### 2.2. Animal subjects

Female *Mecp2*$^{tm1.1Bird}$ mice (Guy et al., 2001) maintained on a pure C57Bl/6J background were used in this study (obtained from Jackson Laboratories, Bar Harbor, ME, or bred in house). These mutant mice were either generated by "in-house" breeding of female *Mecp2*$^{tm1.1Bird}$ mice with wild-type mice on a pure C57Bl/6J background (Jugloff et al., 2008), or they were purchased from Jackson Laboratories directly on this same background. Assays were conducted on female *Mecp2*$^{tm1.1Bird}$ mice at different stages of development (from 3 to 24 months). Wild-type female control mice on the same C57Bl/6J background were similarly generated by "in-house" breeding (Jugloff et al., 2006), or were purchased from Jackson Laboratories on the same genetic background. Some of these female wild-type mice were littermates of the experimental female *Mecp2*$^{tm1.1Bird}$ mice bred "in house". Genotyping was done through polymerase chain reaction (PCR) using primers and conditions described previously (Guy et al., 2001; Jugloff et al., 2008; Maliszewska-Cyna et al., 2010). All animals were raised and housed in a vivarium that was maintained at 22–23 °C with a standard 12-h light on/off cycle that commenced at 6:00 under conditions described previously (Jugloff et al., 2006).

### 2.3. Implantation surgery

Mice were implanted with polyimide-insulated stainless steel microelectrodes (125 μm) as outlined in detail previously (Wu et al., 2008; D'Cruz et al., 2010; Zhang et al., 2016). In brief, mice were anesthetized using 2% isofluorane inhalation, and preconfigured microelectrodes inserted in the somatosensory cortex (Bregma, −0.8 mm; lateral, 1.8 mm; depth, 1.5 mm), and contralateral hippocampal CA1 region (Bregma, −2.3 mm; lateral, 1.7 mm; depth, 2.0 mm). A reference electrode was implanted superficially in the frontal cortex (Bregma, +2.8 mm; lateral, 1.8 mm; depth, 0.5 mm). Following implantation, the mice were allowed to recover from surgery for at least 7 days before experimentation. Electrode positions within the cortex and hippocampus were verified by histological assessments as described previously (Wu et al., 2008; D'Cruz et al., 2010), and by the presence of region-specific and behavioral state appropriate EEG signal activity (Lang et al., 2013; Wither et al., 2013).

### 2.4. EEG recording and analysis

EEG recordings were collected as described previously (D'Cruz et al., 2010; Zhang et al., 2016). In short, the implanted electrodes were connected to a two channel extracellular amplifier with extended head stages (Model-1800; AM Systems Inc., Carlsborg, WA). EEG signals were recorded in a frequency band of 0.1–5000 Hz, amplified 1000x and digitized at 60 KHz (Digidata 1300; Axon Instruments/Molecular Devices, CA). The data were analyzed off-line using pCLAMP software (Axon Instruments/Molecular Devices). All EEG recordings were collected from freely moving mice for different periods of time. For evaluating the progression of epileptiform discharges in young *Mecp2*$^{+/-}$ female mice, the recording sessions were 2 h long on two consecutive days, and taken between 9:00-14:00 to minimize any circadian effects on discharge activity (Wither et al., 2012). The average discharge number from the two days used for analysis. For pharmacological characterization of discharge activity, baseline EEG activities were collected for approximately 30 min before drug administration, and the drug effects evaluated for approximately 1 h following injection. A washout period of at least two days was employed before a subject was administered any other pharmacological compound.

### 2.5. Characterization of epileptiform discharge events

EEG traces were visually inspected to calculate the incidence rate and duration of epileptiform discharge events. Two independent investigators examined the individual data sets, and the individual counts were averaged before entry for final analysis (Zhang et al., 2016). Concordance rates between the two counters were greater than 90%. Epileptiform discharge events were defined as rhythmic spike waveforms, having amplitudes of at least 1.5-fold background, durations of at least 0.5 s, and frequencies between 6 and 10 Hz (D'Cruz et al., 2010; Wither et al., 2012; Zhang et al., 2016). The frequency of discharges was determined by generating spectral plots using the Fast Fourier Transformation (FFT) function in pCLAMP with rectangular binning, 50% window overlap and a spectral resolution of 0.5 Hz.

### 2.6. Drugs and chemicals

General reagents and buffers were obtained from Sigma-Aldrich (Mississauga, Ontario, Canada) unless otherwise stated. The drugs and dosages used in this study were: acetazolamide (X-gen Pharmaceuticals Inc., Big Flats, NY) at doses of 10 mg/kg, 40 mg/kg, and 100 mg/kg, valproic acid (Sigma-Aldrich Canada, Oakville Ontario) at 50 mg/kg; levetiracetam (Sigma-Aldrich) at doses of 100 mg/kg and 300 mg/kg, phenytoin (Sigma-Aldrich) at a dose of 30 mg/kg, carbamazepine (Sigma-Aldrich) at a dose of 12.5 mg/kg, and ethosuximide (Sigma-Aldrich) at a dose of 10 mg/kg. All drugs were dissolved in double distilled $H_2O$ and administered intraperitoneally to the animals.

### 2.7. Statistical analysis

Student's *t*-tests were used for direct comparisons between two normally distributed groups, and Wilcoxon signed-rank tests were used for non-normally distributed groups. For comparisons between multiple groups, one-way ANOVA with Bonferroni post hoc correction for multiple comparisons was used. Significance was set at $p < 0.05$. Mean and standard deviation (SD) are presented throughout the text and figures.

## 3. Results

### 3.1. The discharge phenotype of female Mecp2$^{+/-}$ mice is progressive in nature

We have shown previously that female *Mecp2*$^{+/-}$ mice display