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Research report

Long-term home cage activity scans reveal lowered exploratory behaviour in symptomatic female Rett mice

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HIGHLIGHTS

- *Mecp2*^{Stop} mutant female mice develop Rett-like symptoms late in life (>6 months).
- Symptoms include anomalies in motor, activity and anxiety profiles and were assessed in various behavioural tasks.
- Deficits occurred in ambulatory activity during novelty exploration and habituation to a novel environment.
- Circadian rhythms and anxiety were not affected, but food intake was higher and global activity lower in mutant mice.

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ABSTRACT

Numerous experimental models have been developed to reiterate endophenotypes of Rett syndrome, a neurodevelopmental disorder with a multitude of motor, cognitive and vegetative symptoms. Here, female *Mecp2*^{Stop} mice [1] were characterised at mild symptomatic conditions in tests for anxiety (open field, elevated plus maze) and home cage observation systems for food intake, locomotor activity and circadian rhythms.

Aged 8–9 months, *Mecp2*^{Stop} mice presented with heightened body weight, lower overall activity in the open field, but no anxiety phenotype. Although home cage activity scans conducted in two different observation systems, PhenoMaster and PhenoTyper, confirmed normal circadian activity, they revealed severely compromised habituation to a novel environment in all parameters registered including those derived from a non-linear decay model such as initial exploration maximum, decay half-life of activity and span, as well as plateau. Furthermore, overall activity was significantly reduced in nocturnal periods due to reductions in both fast ambulatory movements, but also a slow lingering. In contrast, light-period activity profiles during which the amount of sleep was highest remained normal in *Mecp2*^{Stop} mice.

These data confirm the slow and progressive development of Rett-like symptoms in female *Mecp2*^{Stop} mice resulting in a prominent reduction of overall locomotor activity, while circadian rhythms are maintained. Alterations in the time-course of habituation may indicate deficiencies in cognitive processing.

1. Introduction

Rett syndrome is a neurological disorder that primarily affects females and is characterised by apparently normal early development up until 6–18 months of age, upon which a period of regression and the development of symptoms including loss of acquired skills, deceleration of head growth, development of ataxia, seizures, scoliosis, breathing anomalies and autistic behaviour is observed [2].

The majority of cases of Rett syndrome result from mutations in the gene encoding methyl-CpG-binding protein 2 (*Mecp2*) on the X chromosome [3]. Several mouse models that lack or express a truncated MeCP2 protein have been developed, and successfully recapitulate many of the clinical symptoms associated with Rett syndrome [4–6]. In keeping with the disease, mutant mice have apparently normal early development followed by the emergence of symptoms and progressive dysfunction such as abnormal gait, hypoactivity, irregular breathing and tremors. Male null mutants present with early onset of symptoms rapidly

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progressing in severity and a short life span of only 8–12 weeks [4,5]. By contrast, heterozygous females show a late-onset phenotype at 4–12 months and, similar to human patients, these symptoms stabilise so that mice experience a normal life span. Truncation of the *Mecp2* allele after codon 308 (*Mecp2*$^{308/Y}$) produces a less severe phenotype and mutant males survive until adulthood [6], but *Mecp2*^{308} heterozygous females have unbalanced patterns of X-chromosome inactivation predominantly expressing the wild-type allele leading to a high degree of variability in phenotypes [7,8].

Neuroanatomical studies of brain areas affected in Rett patients reported a reduction in overall brain volume and atrophy in regions associated with motor function including the caudate nucleus [9] and cerebellum [10,11]. *Mecp2* null mice also display abnormalities in the cerebellum and motor cortex [12] and suggest this to be a mechanism for behaviourally recorded reductions in locomotor activity in the open-field [13–16]. However, it is conceivable that reduced ambulatory activity may not be a direct indicator of locomotion, but instead reflects altered anxiety levels in response to the novel environment, and this would be in agreement with previous studies providing compelling evidence that *Mecp2* deficient mice have heightened levels of anxiety [6,13,17–23]. One possible strategy to monitor locomotor activity uncontaminated of novelty-dependent anxiety would be to perform home cage activity scans in these mice. Such long-term continuous recordings may provide additional information on circadian rhythms, amounts of active locomotion relative to lingering, specific patterns of cage exploration as well as feeding and/or drinking orientated behaviours [24]. It would further reveal whether mouse phenotypes include Rett-typical abnormalities in sleep/wake cycle [25,26] or food intake patterns as a mechanism underlying the observed obesity in patients [27] and *Mecp2* mutant mice [4,5,16,18,19,28].

Despite Rett occurring predominantly in females, investigations using *Mecp2* deficient mouse models have concentrated on males with few exceptions [15,16,20,22]. *Mecp2* null males develop post-natal symptoms early (within few weeks) so that recording can take place in young adult subjects and behavioural endpoints are readily met, with the additional benefit of reduced costs. At the same time, this precludes tracing the behavioural progression in longitudinal analyses as the severity of symptoms rapidly progresses. Consequently, extensive data are available for male *Mecp2*$^{308/Y}$ mice due to their mild phenotypes, but little is known about neurobehavioural anomalies of heterozygous female mice, although females might more realistically mimic the condition of Rett patients at the molecular level [22,29]. Since behavioural phenotypes of Rett models based on different *Mecp2* mutations did not overlap [30], an assessment of each individual model is necessary.

Here, we investigated the anxiety- and motor-related phenotypes of female heterozygous *Mecp2*^{Stop} mice by attempting to capture symptoms in overall activity through scanning their behaviour in an open field followed by fine-grained investigations of circadian/ultradian activity in different home-cage environments. The benefit of using two home-cage observation systems is that in addition to assessing exploratory activity by way of different tracking parameters (infrared beams or overhead infrared camera) each system has distinctive recording features which complement one another and facilitate in the characterisation of the mice in terms of exploration, circadian and feeding orientated behaviour. The PhenoMaster (TSE Systems, Germany) permits direct measurement of food and water intake and also facilitates the distinction between different types of ambulation. Whereas, the PhenoTyper (Noldus IT, The Netherlands) allows the tracking of behaviours (patrolling and feeding) based on user-defined zones, as an indirect measure of feeding behaviour/food intake. We reveal overall reductions in open field exploration due to lower ambulatory activity in *Mecp2*^{Stop} mice not related to heightened anxiety levels or endophenotypes affecting circadian rhythms.

2. Materials and methods

2.1. Subjects

Female mice in which the endogenous *Mecp2* gene was silenced by insertion of a targeted *lox stop* cassette (*Mecp2*^{Stop}) were crossed with males hemizygous for the CreESR transgene [1]. For the purpose of this study only heterozygous *Mecp2*$^{Stop/+}$ females without the cre-ER transgene and wild-type (WT) littermates were selected. Animals were bred on a C57BL6/J/CBA background. Fifty female mice bred at a commercial vendor (Harlan, UK) and delivered to our facility aged 6 months were used and assigned to two cohorts: Cohort 1: $n = 30$ (13 *Mecp2*^{Stop} and 17 WT) were tested in open-field and elevated plus maze; Cohort 2: $n = 20$ (10 *Mecp2*^{stop} and 10 WT) were scanned over 7 days in the PhenoTyper (Noldus IT, Wageningen, The Netherlands) and then over a similar time course in the PhenoMaster (TSE Systems, Bad Homburg, Germany) for circadian activity and food/water intake. Mice were group housed with littermates (unless in the recording equipment) and maintained on a 12 h light/dark cycle (lights on 7 am) with free access to food and water. All procedures were performed in accordance with Home Office regulations.

2.2. Assessment of symptoms

Both body weight and symptom score of mice was recorded weekly; scores for individual symptoms associated with *Mecp2* deficiency included mobility, gait, hindlimb clasping, tremor, breathing and general condition. Each symptom scored as 0 (absent/as wild-type); 1 (symptom present) or 2 (symptom severe) as previously described [1,31]. Testing in the open-field, elevated plus maze and home-cages commenced when the average severity symptom score of *Mecp2*^{Stop} mice was 5/6. At this stage *Mecp2*^{Stop} mice typically presented with a mild/moderate reduction in mobility, abnormal gait, hindlimb clasping and onset of tremors. However, the general health and condition of the mice was comparable to that of WT.

2.3. Open-field test

Explorative behaviour and emotionality was determined in an open field. A white square arena (50 cm × 50 cm) positioned in a dimly lit room (light intensity: 151 lux) with an overhead video camera connected to a PC based tracking software (Ethovision 3.1, Noldus IT) was set up to continuously record activity of the animals. The software monitored the actual movement based on a body-centred contrast subtracted from background and calculated the following parameters: total distance moved; velocity; time spent in inner/outer zones equidistant from the centre of the arena.

All subjects were acclimatised to the room for 5 min, and then released in the centre of the arena for a total exploration time of 10 min. They were returned to their home cages, the arena cleaned with all purpose maceratable wipes, and the next mouse was introduced. Data were analysed using factorial analysis of variance (ANOVA) with genotype (between-subject) and time (within-subject) as factors followed by appropriate post hoc tests with Bonferroni adjustments. In all analyses, the null hypothesis was accepted for alpha smaller than 0.05.

2.4. Elevated plus maze

The elevated plus maze (EPM) was light grey in colour and comprised of two open arms (35 cm × 5 cm) and two closed arms (35 cm × 5 cm × 15 cm; $L \times W \times H$) which extended from a central platform (5 cm × 5 cm). Like arms were arranged opposite to each other in the shape of a plus sign. The apparatus was elevated 42 cm above a table in a dimly illuminated room (light intensity: 142 lux). Prior to testing, mice were transferred to the experimental room and acclimatised for 1 h. At the beginning of a trial mice were individually placed onto the central platform facing an open arm followed by 5 min free exploration. Frequency of entries and time spent in the open/closed arms was recorded online (Ethovision 3.1) and statistically assessed using paired two-tailed *t*-tests. The maze was thoroughly cleaned using ethanol between subjects.

2.5. Home cage activity scans

Two variants of home-cage observation systems were utilised: (1) the PhenoMaster/LabMaster; (2) the PhenoTyper. In both tests, animals were singly housed and subjected to a 12 h light/dark cycle (lights on 7 am, temperature 23 ± 2 °C, relative humidity of 40–60%). They were placed into the cages containing sawdust bedding at lunchtime and given 2 days of habituation before assessment of circadian/ultradian activity over 3 continuous day–night cycles.