Environmental enrichment ameliorates a motor coordination deficit in a mouse model of Rett syndrome – Mecp2 gene dosage effects and BDNF expression

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Abstract
Rett syndrome, commonly associated with mutations of the methyl CpG-binding protein 2 (MECP2) gene, is characterised by an apparently normal early postnatal development followed by deterioration of acquired cognitive and motor coordination skills in early childhood. To evaluate whether environmental factors may influence the disease outcome of Rett syndrome, we tested the effect of environmental enrichment from 4 weeks of age on the behavioural competence of mutant mice harboring a Mecp2<sup>−/−</sup>Tam<sup>−/−</sup> allele. Our findings show that enrichment improves motor coordination in heterozygous Mecp2<sup>+/−</sup> females but not Mecp2<sup>−/−</sup> males. Standard-housed Mecp2<sup>+/−</sup> mice had an initial motor coordination deficit on the accelerating rotarod, which improved with training then deteriorated in subsequent weeks. Enrichment resulted in a significant reduction in this coordination deficit in Mecp2<sup>+/−</sup> mice, returning the performance to wild-type levels. Brain-derived neurotrophic factor (BDNF) protein levels were 75 and 85% of wild-type controls in standard-housed and environmentally enriched Mecp2<sup>+/−</sup> cerebellum, respectively. Mecp2<sup>−/−</sup> mice showed identical deficits of cerebellar BDNF (67% of wild-type controls) irrespective of their housing environment. Our findings demonstrate a positive impact of environmental enrichment in a Rett syndrome model; this impact may be dependent on the existence of one functional copy of Mecp2.

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Here we investigate the effects of EE on hemizygous (Hemi) male and heterozygous (Het) female Mecp2\$^{tm1Tam}\$-null mutant mice (Pelka et al., 2006), and assess whether some degree of MeCP2 expression is required to mediate amelioration of the disease phenotype. Using age-matched WT and mutant littermates, we conducted behavioural tests for motor deficits of clinical relevance to RTT. BDNF protein levels in specific brain regions were also assessed based on the proposed role of this neurotrophin in the pathogenesis of RTT and experience-dependent neuronal plasticity.

Materials and methods

Mice

The Mecp2\$^{tm1Tam}\$ mice (Pelka et al., 2006) were originally bred on a 129 background then crossed to C57BL6 for two generations, followed by breeding amongst offspring of the same generation (F2 intercross) for 3–5 generations with breeder changes. The mixed background was necessary to delay onset of symptoms in males to enable behavioural testing (see Pelka et al., 2006; for details). However, age-matched littermates were used in all experiments to control for possible effects of genetic background unrelated to the Mecp2 mutation (reviewed by Wolfer et al., 2002). After weaning, mice were sent from the Children’s Medical Research Institute in Sydney to the Howard Florey Institute in Melbourne. Animals were maintained in a 12-h light–dark cycle. All behavioural tests were conducted between 08:00 and 18:00 h during the light phase, blind to the genotype. Mice were killed by cervical dislocation on completion of behavioural testing for collection of brain tissue samples. All experiments were approved by the Howard Florey Institute Animal Ethics Committee and carried out in accordance with the requirements of the National Health and Medical Research Council (Australia).

Age and spacing of behavioural tests

The schedule of behavioural tests was designed with allowance for rest periods to minimise stress to the mice (Fig. 1). However, the more rapid onset and progression of symptoms in the males necessitated a shorter interval between tests. The age at which each test was conducted was ± 4 days of the mean group-age for Hemi Mecp2\$^{+/\text{y}}\$-mutant males and ± 7 days for Het Mecp2\$^{+/\text{y}}\$-mutant females.

Housing conditions

At 4 weeks of age, WT and mutant littermates (female Het and male Hemi) were randomly assigned to single-sex standard housing (SH) or EE. EE consisted of larger-sized home cages with nesting material and a variety of objects with differing textures, shapes and sizes, including running wheels (van Dellen et al., 2000; Spires et al., 2004). The objects in the box were changed every 2 days to maintain novelty. Standard mouse boxes contained nesting material only. Animals in both environments were housed communally in groups of five or six. EE commenced at 4 weeks of age and continued for the duration of the study.

Diet supplementation and health monitoring

Food and water were available ad libitum in all boxes. Due to the rapid phenotype onset and consequent decline in health in the males, each box was additionally provided with mashed rodent pellets in containers on the cage floor (Jugloff et al., 2006). Male mice were checked twice weekly for symptoms of disease and animals showing signs of ill health were monitored daily.

Behavioural tests

Accelerating rotarod

Mice were placed on an accelerating rotarod (7650; Ugo Basile, Comerio, VA, Italy) that accelerates from 4 to 40 rpm over 300 s. The latency to fall onto a platform below was recorded. Motor learning was assessed by conducting one session per day for five consecutive days (Crawley, 2007), then comparing the performance on the fifth day to that on the first. Motor coordination was assessed from single rotarod sessions at intervals throughout the study. All rotarod trials were concluded after 300 s. Parameters examined were performance on initial exposure to the rotarod (day 1 of training), change in performance after 5 days of training, and maintenance of performance level.

Female mice. Motor learning was assessed at 12 weeks and coordination at 20, 23, 26 and 29 weeks of age. Motor learning was also assessed in a second female cohort at 6 weeks of age.

Male mice. Motor learning was assessed at 6 weeks and coordination at 7, 8 and 9 weeks of age.
Locomotor activity cell
Mice were placed in a 27 × 27 cm Tru Scan Photobeam Arena (E63–10; Coulbourn Instruments, Allentown, PA, USA) for 30 min to monitor the level of activity in a novel environment. Room lighting was maintained at a dim 8–10 lux to minimize the effects of anxiety on locomotion and exploratory behaviour. Data were recorded using Tru Scan 2.0 software provided by Coulbourn Instruments. Parameters measured were total distance moved, time spent in margins of arena and rearing activity. Locomotor activity was assessed at 15 weeks of age in females and at 6 and 9 weeks of age in males.

Total protein quantitation and BDNF ELISA
Upon completion of behavioural testing, brains from female mice killed at 30 weeks and males at 10 weeks were used to assess BDNF protein levels. Brain tissue was rapidly dissected and frozen at −80 °C. Whole cortex, hippocampus, striatum and cerebellum were sonicated in lysis buffer (Pang et al., 2006) and the DC Protein Assay (BioRad, Hercules, CA, USA) was used to quantify total protein in supernatant. The E-max BDNF ELISA kit (Promega, Madison, WI, USA) was used to calculate total BDNF levels, which were expressed as a proportion of the SH WT control group. The ELISA was validated using recombinant BDNF (Regeneron, Tarrytown, NY, USA).

Statistical analyses
The statistical packages SigmaStat and Prism were used for data analysis. All data was assessed for normal distribution using the D’Agostino and Pearson normality test. Comparisons were made with the SH WT control group and between groups with common environment or genotype factors. Groups were compared using ANOVA with the appropriate number of factors, followed by post hoc pairwise Bonferroni comparisons, or pairwise Mann–Whitney tests in cases of non-normally-distributed data. One-way ANOVA, Wilcoxon or t-tests were used to compare data within groups. Results were considered significant at an α level of 0.05. The rotarod results were analysed using nonparametric statistical tests as the test cut-off point of 300 s generated non-normally-distributed data. In this instance, α of 0.01 was applied for multiple comparisons.

Results

Behaviour: Het female mice
This is the first time that behavioural testing of $\text{Mecp}^{\text{tm1Tam}}$ Het female mice has been reported. EE resulted in a dramatic improvement in the performance baseline and maintenance of motor coordination of female Het mice in the rotarod test for cerebellar motor learning and coordination. Briefly, the mice in this study were randomly allocated to either EE, in which a novel environment was maintained throughout the study duration by the inclusion and regular changing of toys, or SH, in which mice were housed with bedding material only (see Materials and methods for details).

Motor learning
Motor learning was assessed in female mice at 12 weeks of age (for clarity of the text, the results of Mann–Whitney comparisons, α = 0.01, have been placed in Table 1). On initial exposure to the rotarod (day 1; Fig. 2a), SH Het mice had a coordination deficit relative to SH WT and EE Het mice. EE rescued baseline coordination in Het mice, with the time spent on the rotarod by the EE Het matching that of SH and EE WTs. Despite an initial deficit, motor learning ability was preserved in the SH Het mice as their performance improved after the 5-day training period (day 1 vs. 5, Wilcoxon signed-rank test, α = 0.01, P = 0.004). The SH WT group also improved marginally with training (day 1 vs. 5, Wilcoxon signed-rank test, α = 0.01, P = 0.016), and achieved maximal performance by day 5, whilst the EE Het group showed high levels of performance at both day 1 and day 5 (P = 0.156). In both the 12-week and the 6-week test, described below, the EE WT group reached a performance ceiling (300 s) on their first exposure to the rotarod. The EE WT group was therefore unable to improve further and their performance remained constant over the five training days (P = 0.742). After the training period (day 5, Fig. 2a), the SH Het demonstrated a deficit in performance relative only to the SH WT group.

As a coordination deficit was already present in SH Het mice at 12 weeks of age, a second cohort was tested for motor learning only at 6 weeks of age (Fig. 2b). On day 1, the SH Het mice performed as well as the EE Het and SH WT mice (Mann–Whitney test, α = 0.01, SH Het vs. EE Het P = 0.147, SH Het vs. SH WT P = 0.483). After 5 days of training, the four groups performed at a similar level (P > 0.44). The SH Het mice improved their performance over the training period (Wilcoxon signed-rank test, day 1 vs. 5, P = 0.002), whilst the other groups achieved maximal performance (SH WT P = 0.156, EE WT P = 0.383, EE Het P = 0.084).

Motor coordination
Motor coordination was monitored at weeks 20, 23, 26 and 29 (Fig. 2a and Table 1). Although the SH Het mice improved with training, the level of coordination acquired on day 5 was lost in subsequent weeks, with these mice performing significantly worse than the SH WT group at each week of testing. Importantly, EE prevented the progressive coordination deficit from developing in EE Het mice. Coordination in the EE Het mice remained similar to both WT groups for the study.
duration and was superior to SH Het mice until 26 weeks of age. There was no significant difference between the EE and SH Het groups at 29 weeks of age.

**General locomotor activity**

Locomotor activity was assessed in female mice at 15 weeks of age. A two-way ANOVA of distance moved (Fig. 3a) indicated that EE decreased locomotion ($F_{1,43} = 7.35, P < 0.01$) but that genotype had no effect ($F_{1,43} = 1.48, P = 0.231$). Post hoc Bonferroni tests showed that although there was no difference in distance travelled between the two EE groups ($t = 2.07, P = 0.267$), the EE Het mice moved less than SH WT littermates ($t = 2.8, P = 0.045$) and also less than SH Het mice ($t = 3.1, P = 0.021$). This may be an EE-induced change in exploratory behaviour enhanced in mice with the Mecp2 Het mutation. Enrichment was recently shown to alter exploration in WT and mutant mice in other behavioural tests (Nithianantharajah et al., 2008). Time spent in the arena margin, which can be a measure of anxiety-like behaviour, did not differ between groups (Fig. 3b; two-way ANOVA, $F_{1,43} < 1.9, P > 0.18$). Rearing behaviour, as assessed by vertical plane entries, was analysed with Mann–Whitney pairwise comparisons due to its non-normal distribution (Fig. 3c). The two Het groups reared significantly less than the WT groups (SH Het vs. SH WT $P = 0.045$, EE Het vs. SH WT $P = 0.008$, EE Hemi vs. EE WT $P = 0.018$). There was no effect of EE on this behaviour.

**Behaviour: Hemi male mice**

In initial studies with the Mecp2\textsuperscript{Hemi}\textsuperscript{Tam} model of RTT, Hemi mutant males were shown to have a motor coordination deficit and a cerebellar motor learning deficit (Pelka et al., 2006) corresponding to the ataxia and apraxia observed in RTT patients. The coordination deficits were confirmed and extended in the current study, with Hemi mice showing poor coordination and inability to improve performance on the rotarod without displaying overt differences in floor plane movement in a novel environment. Housing the male mice in EE did not ameliorate the motor phenotype.

**Motor learning and coordination**

On first exposure of the male mice to the rotarod at 6 weeks of age (day 1; Fig. 4), the EE WT animals showed better performance than the SH WT group (Mann–Whitney test, $P = 0.0006$). The EE Hemi mice displayed reduced performance relative to the EE WT mice on day 1 (Mann–Whitney tests, $P = 0.01$, $P = 0.001$) but not to SH WT ($P = 0.689$). The SH Hemi were also similar to SH WT on day 1 (Mann–Whitney test, $P = 0.01$, $P = 0.034$). On day 5 (Fig. 4), upon completion of training, there was no difference in performance between the EE and SH WT, with both groups remaining for the maximum 300 s on the rotarod (Mann–Whitney test, $P = 0.243$). SH WT males showed significant improvement between days 1 and 5 as a result of training (Wilcoxon signed-rank test, $P = 0.004$), whereas the EE WT had already reached the performance ceiling on their first exposure to the test and therefore maintained their performance without further improvement (Wilcoxon test, $P = 0.688$). Both Hemi groups, however, remained inferior in performance on day 5 compared to the relevant WT groups (Mann–Whitney tests, $P = 0.01$, SH WT vs. SH Hemi $P = 0.003$, SH WT vs. EE Hemi $P = 0.0004$, EE WT vs. EE Hemi $P = 0.001$). Neither of the Hemi groups improved performance with training, indicating impaired motor learning, and EE had no effect (Wilcoxon test day 1 vs. day 5: SH Hemi $P = 0.625$, EE Hemi $P = 1.00$). Continued assessment of motor coordination from 7 to 9 weeks of age showed persistent impairment in the Hemi groups (data not shown due to low numbers).
Locomotor activity was measured at 6 and 9 weeks of age as an indicator of general activity levels. Impaired locomotion resulting from progression of disease symptoms may alter the outcome of behavioural tests; hence locomotor activity was assessed before and after the remainder of the behavioural testing series. Figure 5a shows that at 6 weeks of age there was no effect of genotype or environment on the total distance travelled, indicating that the Hemi mice were capable of adequate movement for exploration (two-way ANOVA, F1,25 < 3.8, P > 0.06). There was also no difference in time spent in the margins of the activity cell (Fig. 5b, two-way ANOVA, F 1,25 < 0.45, P > 0.508). Rearing activity (Fig. 5c), however, varied between genotypes. The SH Hemi mice reared significantly less than their control WT littermates, (nonparametric distribution; pairwise Mann–Whitney comparisons, SH Hemi vs. SH WT \( P = 0.029 \)). No rearing was recorded for the EE Hemi group, which precluded statistical analyses. There was no effect of environment on rearing in the WT mice (Mann–Whitney test, \( P = 0.968 \)). A similar pattern was observed for all three movement parameters for animals surviving at 9 weeks of age (data not shown).

**BDNF protein levels in brain tissues**

Changes in BDNF protein levels in cortex, striatum, cerebellum and hippocampus of the female mice were determined at 30 weeks of age (Fig. 6a–d). BDNF expression was also assessed in the cerebellum of male mice at 10 weeks of age (Fig. 7). Briefly, brain regions were sonicated and the supernatant collected, then total BDNF protein was assayed using a BDNF ELISA kit. BDNF protein content of the SH Het, EE Het and EE WT groups were expressed as a percentage of that of the SH WT control group (Chang et al., 2006).

For the female mice, the BDNF concentration in the cerebellum showed a significant effect of genotype (Fig. 6a; two-way ANOVA, \( F_{1,25} = 24.52, P < 0.001 \)) but not environment (\( F_{1,25} = 0.124, \ P = 0.27 \)). Post hoc Bonferroni tests showed thatEE Het traveled less than SH WT and SH Het groups (\( P < 0.05 \)) but were similar to EE WT (\( P = 0.27 \)). (b) Time spent in margin zone, no significant difference between groups (two-way ANOVA). (c) Vertical plane entries. Both Het groups showed significantly fewer vertical entries than did relevant WT groups (\( P < 0.05 \) SH Het vs. SH WT; \( P < 0.05 \) EE Het vs. SH WT and EE WT; Mann–Whitney tests). Numbers included: SH WT, 11; EE WT, 12; SH Het, 11; EE Hemi, 13. Values are mean ± SEM.
were found in the cortex (Fig. 6c; \( F_{1,26} < 1.4, P > 0.25 \)) or striatum (Fig. 6d; \( F_{1,26} < 1.8, P > 0.57 \)).

In the male cerebellum there was a significant effect of genotype, with Hemi mice having a significantly lower BDNF concentration (Fig. 7; two-way ANOVA: \( F_{1,19} = 69.32, P < 0.001 \)). Bonferroni post hoc tests indicated that both SH and EE Hemi groups displayed a deficit in cerebellar BDNF relative to WT groups (SH WT vs. SH Hemi: \( t = 5.45, P < 0.001 \); SH WT vs. EE Hemi: \( t = 6.16, P < 0.001 \); EE WT vs. EE Hemi: \( t = 6.35, P < 0.001 \)).

Discussion

This study documents a marked rescue in motor coordination and a partial normalization in cerebellar BDNF expression of Het female MeCP2\( ^{m1Tam} \) mice, a model of RTT, resulting from exposure to EE.

There are several mouse models of RTT (Chen et al., 2001; Guy et al., 2001; Shahbazian et al., 2002; Collins et al., 2004; Pelka et al., 2006) which recapitulate aspects of the clinical phenotype found principally in human females Het for MECP2 mutations. The majority of mouse studies have been conducted on male Hemis which display a more severe phenotype than that of Het female mice, which harbour both normal and MeCP2-deficient cells due to X-chromosome inactivation (Young & Zoghbi, 2004; Watson et al., 2005). Male mice have a phenotype progression reminiscent of, although more severe than, RTT patients and provide insight into the outcome of total loss of MeCP2 function. However, the existence of one MeCP2 copy, and therefore some level of MeCP2 function, in the female MeCP2\(+/-\) mice is closer to human RTT at the molecular level (reviewed by LaSalle, 2004; Stearns et al., 2007). We opted to investigate both Hemi and Het models of RTT for this reason.

In the current study MeCP2\(+/-\) mice exhibited deficits in motor coordination which worsened progressively, developing an apparent motor coordination deficit at 12 weeks of age but not at 6 weeks. Housing these mice in an environment that stimulates sensory, motor and cognitive activity reversed the deficit in motor coordination. The EE Hets maintained their performance near WT levels up to 29 weeks of age, whilst the SH Hets maintained their performance near WT levels up to 29 weeks of age, whilst the SH Hemi mice showed significantly fewer vertical entries than did WT groups (*\( P < 0.05 \), SH Hemi vs. SH WT; Mann-Whitney tests). Numbers included: SH WT, 9; EE WT, 10; SH Hemi, 5; EE Hemi, 5. Values are mean ± SEM.

FIG. 5. Male locomotor behaviour at 6 weeks of age. (a) Distance traveled, no significant difference among groups (two-way ANOVA). (b) Time spent in margin zone, no significant difference between groups (two-way ANOVA). (c) Vertical plane entries. EE Hemi group showed no vertical plane entries. SH Hemi showed significantly fewer vertical entries than did WT groups (*\( P < 0.05 \), SH Hemi vs. SH WT; Mann-Whitney tests). Numbers included: SH WT, 9; EE WT, 10; SH Hemi, 5; EE Hemi, 5. Values are mean ± SEM.
study, is thought to mimic stereotypic hand-wringing in RTT patients. As noted by Chang et al. (2006), this phenotype is very similar in appearance to the hind-limb clasping displayed by Bdnf conditional mutant mice, and it has been speculated that a BDNF deficit is involved in this behaviour in both mutants. At the final timepoint examined in the present study, the hind-limb clasping phenotype was apparent in 37.5% of EE Het and 55.5% of SH Het mice (data not shown).

Analysis of BDNF expression in the female mice indicated that BDNF protein levels were reduced to 75% of SH WT levels in the cerebellum of the SH Het (Fig. 6a), which is informative in the context of the impaired motor coordination observed in these animals. Enrichment raised mean cerebellar BDNF to 85% of SH WT levels. Although the EE Het group expressed levels of BDNF in the cerebellum that were statistically indistinguishable from the SH WT levels, the increase in BDNF in the Het mice with EE did not achieve statistical significance between EE Het and SH Het mice. Therefore, at the relatively old age of 30 weeks, there was only partial normalization of BDNF expression in the EE Het mice. This molecular result correlates with the rotarod performance of the Het mice at 29 weeks of age (Fig 2a), just prior to tissue collection, when performance was starting to decline.

Analysis of BDNF in the male mice revealed a deficit in the cerebellum in the SH Hemi mice, at 67% of SH WT levels. Significantly, cerebellar BDNF levels were also 67% of SH WT controls in the EE Hemi mice with EE did not achieve statistical significance between EE Het and SH Het mice. Therefore, at the relatively old age of 30 weeks, there was only partial normalization of BDNF expression in the EE Hemi mice. This molecular result correlates with the rotarod performance of the Het mice at 29 weeks of age (Fig 2a), just prior to tissue collection, when performance was starting to decline.

Analysis of BDNF expression in the cerebellum of male WT and Hemi mice, expressed as % of SH WT. Significant effect of genotype (two-way ANOVA, P < 0.001). ***P < 0.001, SH Hemi vs. SH WT and EE Hemi vs. EE WT (post hoc Bonferroni tests). Numbers included: SH WT, 9; SH Hemi, 3; EE WT, 7; EE Hemi, 4. Values are mean ± SEM.

![Fig. 6. BDNF expression in the female WT and Het mice, expressed as % of SH WT. (a) Cerebellum: significant effect of genotype (two-way ANOVA, P < 0.001). SH WT vs. SH Het, ***P < 0.01; EE WT vs. EE Het, *P < 0.05 (post hoc Bonferroni tests). There were no differences between SH Het and EE Het or SH WT and EE Het. (b) Hippocampus: significant effect of environment (two-way ANOVA, P < 0.05), post hoc tests were not significant. (c) Cortex: no variation with genotype or environment. (d) Striatum: no variation with genotype or environment. Numbers included: SH WT, 8; SH Het, 7; EE WT, 8; EE Het, 7. Values are mean ± SEM.](image1)

![Fig. 7. BDNF expression in the cerebellum of male WT and Hemi mice, expressed as % of SH WT. Significant effect of genotype (two-way ANOVA, P < 0.001). ***P < 0.001, SH Hemi vs. SH WT and EE Hemi vs. EE WT (post hoc Bonferroni tests). Numbers included: SH WT, 9; SH Hemi, 3; EE WT, 7; EE Hemi, 4. Values are mean ± SEM.](image2)
lifespan and reduces the locomotor deficit (Chang et al., 2006). Therefore, the beneficial effect of EE we observed in the Mecp2+/− model of RTT may be due, at least partially, to upregulation of BDNF. It is noteworthy that this behavioural response was achieved by a relatively moderate form of home-cage EE (for review of published enrichment protocols used in rodent models of brain disorders, see Nithianantharajah & Hannan, 2006). A study in male Mecp2-knockout mice reported a significant decrease in BDNF levels at 6–8 weeks of age, with knockouts expressing only 79% of the BDNF protein level of WT mice in the cortex and 59% in the cerebellum (Chang et al., 2006). Our results in the SH Hemi cerebellum are similar to these. Furthermore, the percentage change we observed in the Ht cerebellum was milder than that in the Hemi null mice and correlates with the coordination differences observed between the models. The lack of BDNF deficit in the cortex of the Mecp2−/− mice is not surprising either, as Wang et al. (2006) observed no difference in cortical or hippocampal BDNF protein levels in null male mice despite significant deficits in the brainstem and nodose ganglia. Here, a significant effect of EE on hippocampal BDNF was observed in the female mice irrespective of genotype. EE is known to induce neural plasticity, including enhancing adult hippocampal neurogenesis and synaptic plasticity (Kempermann et al., 1997) and increasing neurotrophin expression (reviewed by van Praag et al., 2000). As our data are consistent with actions on the cerebellum, molecular changes underlying experience-dependent synaptic plasticity will be of interest in future investigations. This could include other trophic factors and neurotransmitter receptors, as well as various pre synaptic and postsynaptic signaling proteins.

Although it is difficult to make direct comparisons between the male and female cohorts used in this study due to differences in the duration of EE, age of tissue collection and stage of disease, the results are suggestive of the importance of Mecp2 gene copy number in the response to EE, and not just phenotypic expression. The effect of EE on the Mecp2+/− mice is therefore more likely to be mediated by cells expressing an active WT Mecp2 allele. Further significance of Mecp2 gene copy number in disease progression and potential for recovery of symptoms can be gleaned from recent studies documenting amelioration of RTT-like symptoms in animal models with restoration of Mecp2 expression (Giacometti et al., 2007; Guy et al., 2007). Of note in these studies was a significant gene dosage effect, with some degree of phenotypic improvement in transgenic lines with only 10% of cells expressing Mecp2 (Giacometti et al., 2007). Collins et al. (2004) also showed the importance of tight regulation of MeCP2 levels in vivo, with mice expressing double the normal WT dose displaying disease symptoms with increasing age. Additionally, as MeCP2 levels influence excitatory synaptic strength via regulation of glutamatergic synaptic density (Chao et al., 2007), the presence of some level of functional MeCP2 in the females (dependent on X-chromosome inactivation patterns) may afford the later onset and milder presentation, hence some capacity to respond to treatments including EE. As neuronal activity-dependent phosphorylation of MeCP2 appears to regulate dendritic patterning, spine morphogenesis and BDNF transcription (Zhou et al., 2006; reviewed by Chahrour & Zoghbi, 2007) it is plausible that, in females, MeCP2 function may be upregulated by EE, reversing part of the phenotype.

The results of our study suggest that symptomatic alleviation of RTT may be achieved by enhanced sensory, cognitive and motor stimulation, underscoring the notion that EE should be actively incorporated into the treatment paradigm for RTT. Furthermore, molecular insights into these beneficial effects of EE may facilitate future development of new therapeutic approaches for RTT and other brain disorders.

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Abbreviations

BDNF, brain-derived neurotrophic factor; EE, environmental enrichment; Hemi, hemizygous; Het, heterozygous; MeCP2, methyl CpG-binding protein 2; RTT, Rett syndrome; SH, standard housing; WT, wild-type.

References


