Supplemental Material

Abstract

Rett syndrome (RTT) is a post-natal neurological disorder caused by mutations in *MECP2*, encoding the epigenetic regulator Methyl-CpG-Binding Protein 2 (MeCP2). The onset of RTT symptoms during early life together with findings suggesting neurodevelopmental abnormalities in RTT and mouse models of RTT raised the question of whether maintaining MeCP2 function exclusively during early life might protect against disease. We show using an inducible model of RTT that deletion of *Mecp2* in adult mice recapitulates the germline knock-out phenotype, underscoring the ongoing role of MeCP2 in adult neurological function. Moreover, unlike the effects of other epigenetic instructions programmed during early life, the effects of early MeCP2 function are lost soon after its deletion. These findings suggest that therapies for RTT must be maintained throughout life.

Materials and Methods

Materials

Animals. *Mecp2*^{flox} mice *(3)* and *Mecp2*^{null} mice *(3)* were maintained on pure 129/SvEvTac backgrounds, and CAGGS-CreER (CreER) mice *(4)* were maintained on a pure C57BL/6J background. For all experiments, only male F1 hybrid (129.B6 background) mice were used. All research and animal care procedures were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee

Tamoxifen preparation. Tamoxifen citrate (Sigma) was prepared to 20mg/mL in corn oil (Sigma) by sonication, aliquotted and frozen at -20ºC until use.

Methods

Tissue processing. Tissue used for molecular studies was dissected on ice, flash-frozen in liquid nitrogen and stored at -80ºC before further processing. Frozen tissue was later homogenized in Trizol (Invitrogen) using a Polytron homogenizer; DNA, RNA, and protein were isolated according to manufacturer's instructions.

Western blot. Performed as previously described*(8)*. Primary antibodies used include rabbit anti-MeCP2 (generated in Zoghbi lab, #0535), and rabbit anti-pan histone H3 (Millipore).

Immunofluorescence. Performed as previously described *(8-9)*. Sections were cut at 30um on a cryostat (Leica CM3050 S), and stained with primary antibody (rabbit, anti-MeCP2, Cell Signalling) and secondary antibody (goat anti-rabbit Alexa555). DAPI was used to stain DNA/nuclei. Images were acquired on Leica TCS SP5 laser-scanning confocal microscope.

Phenotyping. All mice from *Mecp2*^{flox} x CreER cohorts were dosed (double and single mutants, and wild-type) starting at PND60, while germline KO (*Mecp2*^{null}) cohorts were not treated. Assays for symptoms and behavior were performed blinded to genotype and as described in previous work*(2,8-9)*. Ages at testing for adult *Mecp2* KO mice were: symptoms (23 weeks; 10 weeks after dosing), footslip/rotarod (20 weeks; 7 weeks after dosing), nesting (17 weeks; 4 weeks after dosing), and conditioned fear (18 weeks; 5 weeks after dosing). For germline *Mecp2* KO mice: symptoms (10-11 weeks), footslip/rotarod (6-7 weeks), nesting (5-6 weeks), conditioned fear (6-7 weeks). Data are shown as mean ± s.e.m. and analyzed by two-way ANOVA (genotype x cohort) followed by Fisher's least significant difference (l.s.d.) post hoc test, unless otherwise stated.

Symptom score. Scoring was performed as described in Guy *et al.(2)*. Briefly, mice were evaluated for tremor, gait abnormalities, immobility, hind-limb clasping, breathing, and general condition, and a score (0 – 2, 0 = not present, 1 = present, 2 = present and severe) assigned for each symptom. The sum total (0 – 12) was used as the symptom score for each mouse.

Grid walk. Mice were placed on a wire grid (17 inch × 11 inch, 1-cm^2 spacing, 6 inch elevation) and the number of footslips was manually scored over 5 minutes while distance traveled was recorded by a VersaMax Activity monitor using photobeam breaks*(5)*.

Rotarod. This assay was performed as previously described*(8)*. Mice were placed on the accelerating rotarod apparatus (Ugo Basile) for 4 trials (four trials a day on two consecutive days) of 5 minutes each, during which the rod accelerated from 4-40 rpm. The amount of time for each mouse to fall was recorded for each trial. Rotarod data presented is from average performance on day 2.

Nesting. Mice were singly housed with a single folded Kimwipe overnight and the degree of nest building was assessed on a scale of 0 – 3 (0 = material virtually untouched, 3 = completely shredded).

Conditioned fear. This test was performed as previously described*(9)* over two days. Briefly, mice were trained on Day 1 to associate a white noise sound pulse ("cue") with a subsequent electric shock in a specialized chamber (Actimetrics). On Day 2, cue-dependent memory was assessed by measuring freezing in response to the cue in the same chamber following alteration of contextual clues. Data were analyzed by two-way ANOVA (genotype x cohort) followed by Fisher's l.s.d. test.

RT-QPCR. For gene expression studies, mice were dosed starting at 3-4 months of life and the hypothalamus was dissected as previously described *(5)* 11 weeks after dosing. RNA was isolated from Trizol, processed using RNeasy Cleanup Kit (Qiagen) and cDNA synthesis performed using SuperScript III RT (Invitrogen). QPCR was performed using custom primers and Quanta PerfeCTa SYBR Green Fast Mix on a CFX96 Real-Time PCR detection system with C1000 thermal cycler (Biorad). Results were quantified using the standard curve method and normalized to detected levels of *S16*. Statistical significance was calculated using one-way ANOVA (genotype) and Fisher's l.s.d. post-hoc test.

QPCR. Sagittal hemisections of whole brain tissue were collected from mice 30 days after the termination of dosing. QPCR detection of the *Mecp2* exonic region to be deleted was performed as described above, using custom primers.