Neuroscience 146 (2007) 907–921

BEHAVIORAL AND ANATOMICAL ABNORMALITIES IN *MECP2* MUTANT MICE: A MODEL FOR RETT SYNDROME

N. A. STEARNS, L. R. SCHAEVITZ, H. BOWLING, N. NAG, U. V. BERGER AND J. BERGER-SWEENEY*

Department of Biological Sciences, Wellesley College, 106 Central Street, Wellesley, MA 02481, USA

Abstract—Over 90% of Rett syndrome (RTT) cases have a mutation in the X-linked gene encoding methyl CpG binding-protein 2 (MeCP2). A mouse model that reprises clinical manifestations of the disease would be valuable for examining disease mechanisms. Here, we characterize physical and behavioral measures, as well as brain region volumes in young adult mice that have mutations in mouse methyl CpG binding-protein 2 gene (*Mecp2*) to serve as a baseline for other studies. Hemizygous males, which produce no functional protein, exhibit hypoactivity and abnormalities in locomotion, stereotypies, and anxiety reminiscent of the clinical condition. The mutant males also exhibit cognitive deficits in fear conditioning and object recognition relative to wildtypes. Volumetric analyses of male brains revealed a 25% reduction in whole brain volume in mutants relative to wildtypes; regional differences were also apparent. Mutants had decreased volumes in three specific brain regions: the amygdala (39%), hippocampus (21%), and striatum (29%). Heterozygous females, which produce varying amounts of functional protein, displayed a less severe behavioral phenotype. The mutant females exhibit abnormalities in locomotion, anxiety measures, and cognitive deficits in object recognition in an open field. This study provides the first evidence that the abnormal motor and cognitive behavioral phenotype in *Mecp2* mice is consistent with specific volume reductions in brain regions associated with these behaviors, and shows how these data parallel the human condition. The *Mecp2* mutant mice provide a very good model in which to examine molecular and behavioral mechanisms, as well as potential therapeutic interventions in RTT.

Key words: zero maze, swim maze, fear conditioning, AMIRA, volumetric analysis, object recognition.

Rett syndrome (RTT), a major cause of mental retardation in females, is a disorder with a broad array of clinical manifestations (Hagberg, 1985; Hagberg et al., 1999). Four stages of classic RTT include 1) relatively normal development until 6–18 months of age, when head growth slows down, 2) rapid regression at 1–3 years, when acquired skills are lost and stereotypic movements, mental retardation, and emotional disturbances become apparent, 3) pseudo-stabilization at 5–10 years, when autistic symptoms lessen but mental retardation and stereotypies persist, and breathing abnormalities develop. After stabilization, which can last until adulthood, 4) osteoporosis, scoliosis, and dystonia may develop. The median age of death in one study was 24 years; however, when nutritional requirements are met and physical therapies applied, most RTT females survive longer (Schneider and Glaze, 2002).

Magnetic resonance imaging (MRI) studies and postmortem analysis of RTT individuals suggest that these clinical manifestations are correlated with underlying anatomical changes. Volumetric MRI studies of RTT brains and age-matched controls show that on average, there is an overall reduction in whole brain size; however, this reduction does not appear to be uniform (Casanova et al., 1991; Reiss et al., 1993; Armstrong, 2005). The brain regions most significantly affected in RTT individuals appear to be associated with the two most prominent clinical features of RTT: impaired motor function and impaired higher cognitive processes. Voluntary motor function is regulated through the basal ganglia circuit connecting cortex to striatum and thalamus. Both the cortex and caudate nucleus of the striatum are reduced in RTT individuals (Reiss et al., 1993; Takakusaki et al., 2004). Coordination of movement and fine motor control are putatively regulated by the cerebellum, which is also reduced in RTT individuals (Murakami et al., 1992; Mauk et al., 2000). Brain regions involved in higher cognitive functions are also affected in RTT individuals; the prefrontal cortex, in addition to the striatum, is reduced (Reiss et al., 1993). Additionally, the white matter tract bridging the two cerebral hemispheres, the corpus callosum, has also been shown to be reduced in some cases (Gotoh et al., 2001). However, in general it appears that gray matter is affected more than white matter (Casanova et al., 1991; Reiss et al., 1993; Naidu et al., 2001).

Mutations of human methyl CpG binding-protein 2 gene (*MECP2*), an X-linked gene encoding methyl CpG binding-protein 2 (MeCP2), are documented in over 90% of sporadic RTT cases (Williamson and Christodoulou, 2006). MeCP2 is a ubiquitously located transcription repressor that affects gene transcription rates by binding methylated DNA, changing chromatin structure and rendering it transcriptionally inactive (Wan et al., 2001). Once

*Corresponding author. Tel: +1-781-283-3050; fax: +1-781-283-3642.
E-mail address: jbergers@wellesley.edu (J. Berger-Sweeney).

Abbreviations: ANOVA, analysis of variance; DO, displaced object in object recognition task; Fhetero, female *Mecp2* heterozygous mice; Fwt, female wildtype mice; Mecp2, mouse methyl CpG binding-protein 2; *Mecp2*, mouse methyl CpG binding-protein 2 gene; MeCP2, human methyl CpG binding-protein 2; *MECP2*, human methyl CpG binding-protein 2 gene; Mnull, male *Mecp2* null mice; MRI, magnetic resonance imaging; Mwt, male wildtype mice; NDO, non-displaced object in object recognition task; NSO, non-substituted object in object recognition task; PBS, phosphate-buffered saline; PD, postnatal day; PFA, paraformaldehyde; RTT, Rett syndrome; SO, substituted object in object recognition task.

0306-4522/07$30.00+0.00 © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2007.02.009

thought that only females with the mutation survive, it appears that males with mutations in their single *MECP2* gene often present with a more severe phenotype (reviewed in Bienvenu and Chelly, 2006). It is believed that the wide range of symptom severities in both males and females is due both to the large number of documented mutations in *MECP2* (over 2000), as well as random X chromosome inactivation in females. Random X inactivation would result in relatively more or fewer cells expressing normal MeCP2 protein (Webb and Latif, 2001; Naidu et al., 2003; Bienvenu and Chelly, 2006).

The precise role of MeCP2 in producing the clinical RTT phenotypes is still unclear. Experiments examining the spatial and temporal expression pattern of MeCP2 protein suggest that MeCP2 may play an important role in controlling neuronal maturation through the regulation of synapse formation and maintenance (Mullaney et al., 2004; Kaufmann et al., 2005). MeCP2 expression parallels the timing of post-migration differentiation in neurons and appears to play a prominent role in maturation of cortex, hippocampus, cerebellum, thalamus, and striatum, many of the same regions, which show significant volumetric reductions in RTT (Shahbazian et al., 2002; Mullaney et al., 2004; Kaufmann et al., 2005). In these regions, the absence of the appropriate levels of MeCP2, as seen in RTT, result in shorter, underdeveloped dendritic arborizations and reduced neuronal size as a consequence of inappropriate synaptic maturation. Reduced dendritic arborization and neuronal size are believed to account for decreases in brain volume (Bauman et al., 1995). Recent evidence suggests that MeCP2 may play a role in synapse maturation and maintenance of appropriate dendritic arborization by regulating activity-associated expression of BDNF in neuronal cells (Chen et al., 2003). Interestingly, the timing of the onset of symptoms in humans and the regression of learned behaviors suggest that disruption of normal synaptic maturation and stabilization of dendritic arborization underlie the abnormal motor and cognitive phenotype in this disorder (Johnston et al., 2001; Zoghbi, 2003).

The potential benefit of animal models that reprise symptoms of RTT is enormous, and has led to the creation of several mouse models expressing alterations in mouse methyl CpG binding-protein 2 (Mecp2). Four mouse models currently exist. Three models result in the loss of functional Mecp2 protein either through the deletion of exon 3 of the *Mecp2* gene (*Mecp2*^{1lox}, Chen et al., 2001) or by the deletion of exons 3 and 4 (*Mecp2*$^{tm1\text{-}1Bird}$, Guy et al., 2001; *Mecp2*^{tm1Tam}, Pelka et al., 2006). In the fourth model, the Mecp2 protein is truncated after codon 308 (*Mecp2*^{308}), retaining several key functional domains (Shahbazian et al., 2002). In all four models, mutants exhibit motor abnormalities; however, only a small subset of cognitive tasks has been performed in the *Mecp2*^{tm1Tam} and *Mecp2*^{308} mouse models (Moretti et al., 2006; Pelka et al., 2006). To our knowledge a battery of cognitive tasks has not been performed on a single animal model of RTT, nor have researchers attempted to associate motor, anxiety, and cognitive impairments with the anatomical changes in the brain which are also hallmarks of RTT. The *Mecp2*^{1lox} mouse model of RTT, utilized for these studies, allowed us to determine the effects of the loss of *Mecp2* function on both behavior and anatomy without the added complication of determining the functional consequence of a truncated Mecp2 protein. Additionally, the severity of symptoms previously described in the *Mecp2*^{1lox} model, both onset of symptoms and lifespan, appeared more reminiscent of RTT in humans than other available mouse models.

Here we describe the phenotype of *Mecp2*^{1lox} mice of both sexes between 4 and 12 weeks of age including motoric, cognitive and anxiety-related abnormalities. Both male hemizygous and female heterozygous (Fhetero) mice were used for all behavioral studies in contrast to most other studies which examine only male mice. We used male hemizygous animals for reasons similar to those cited by other researchers: male hemizygous mice represent completely null animals (no Mecp2 protein) and random X-inactivation leaves an unpredictable proportion of X-chromosomes carrying the Mecp2-null allele in the female making statistical comparisons potentially difficult. Despite this last drawback, we decided to use Fhetero animals also because most research in humans studies female patients with RTT, and we found that the motor impairment in male mutants made some physically demanding cognitive tasks difficult to interpret. In addition in the current study, we examine brain volumes in regions associated with the specific behavioral tasks. Because the most pronounced and reliable motoric and behavioral changes were in the mutant males, we examined brain volumes, only in males, at 5 weeks of age. We measured the volumes of several regions, focusing on regions associated with the RTT abnormal behavioral phenotype such as amygdala, cerebellum, hippocampus, somatomotor cortex, striatum, and thalamus as well as the white matter tracts the anterior commissure and corpus callosum.

EXPERIMENTAL PROCEDURES

Subjects

Mecp2^{1lox} mice were generated as described previously (Chen et al., 2001). Two female founder mice heterozygous for the *Mecp2*^{1lox} null allele were obtained from Dr. R. Jaenisch (Massachusetts Institute of Technology, Cambridge, MA, USA) and used to establish a colony of *Mecp2*^{1lox} animals in the Department of Biological Sciences, Wellesley College. These heterozygous females of mixed genetic background (primarily BALB/C with some 129 and C57BL/6) were mated to wildtype C57BL/6J males; the Fhetero offspring were mated to wildtype C57BL/6J males. All experiments were conducted on *Mecp2*^{1lox} mice, back-crossed for more than eight generations, and their wild-type littermates. Mice were maintained on a 12-h light/dark cycle with lights on at 07:00 h, and food and water available *ad libitum* except during behavioral testing. All procedures were approved by the Wellesley College Institutional Animal Care and Use Committee (IACUC) and conformed to the standards set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All attempts were made to reduce the number of animals used and to prevent their suffering.