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Neonatal exposure to low dose corticosterone persistently modulates hippocampal mineralocorticoid receptor expression and improves locomotor/exploratory behaviour in a mouse model of Rett syndrome

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ABSTRACT

Rett syndrome (RTT) is a pervasive neurodevelopmental disorder, primarily affecting girls. RTT causes a wide variety of debilitating symptoms and no cure currently exists. Mouse models bearing mutations in the *Mecp2* gene recapitulate most physiological and behavioural RTT-related abnormalities. Stimulating neonatal environments (e.g. brief maternal separations or maternal low-dose corticosterone supplementation) reduce stress and fear responses at adulthood. The present study investigated whether impacting early in development the hypothalamic-pituitary-adrenal axis, by exposing Mecp2-308 mutant pups to a low dose of corticosterone (50 μg/ml, during the 1st week of life) may contrast RTT-related abnormalities in neuroendocrine regulation and behavioural adaptation at adulthood. In line with previous reports, when fully symptomatic, MeCP2-308 mice showed a reduction in the regular nocturnal hyperactivity in the home-cage and increased anxiety-like behaviours and plasma corticosterone (CORT) levels in response to restraint stress. An abnormal elevation in mRNA levels of mineralocorticoid receptors (*MR*) and *BDNF* gene was also evident in the hippocampus of fully symptomatic mutant mice. Neonatal CORT modulated *MR* gene expression and behavioural reactivity towards a novel object, also restoring wt-like levels of locomotor/exploratory behaviour in mutant mice. Enhanced sensitivity to the neonatal treatment (in terms of increase in GR and MR mRNA levels), was also evident in the hippocampus of MeCP2-308 mice compared to wt littermates. Present results corroborate the hypothesis that targeting the glucocorticoid system may prove valid in contrasting at least some of the RTT-related symptoms and provide evidence that pharmacological interventions during critical early time windows can persistently improve the behavioural phenotype of RTT mice. Current data also support the emerging role played by Mecp2 in mediating the epigenetic programming induced by early life events and indicate that, in the absence of functional MeCP2, programming of the central nervous system in response to early environmental stimuli is abnormally regulated.

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1. Introduction

Classified together with autism into the DSM-IV in the group of pervasive developmental disorders, Rett syndrome (RTT) is a rare neurodevelopmental disorder and a genetic cause of intellectual disability, primarily affecting girls with a prevalence of 1:10,000 births (Chahrour and Zoghbi, 2007). One essential feature of RTT is the apparently normal perinatal development until about 6–18 months of age, when RTT patients start loosing their acquired cognitive, social, and motor skills and develop a wide variety of symptoms, including autistic-like behaviours, sleep disturbances, stereotypic hand movements and severe mental retardation (Hagberg, 2002).

Mutations in the methyl–CpG–binding protein 2 gene (*MECP2*) are found in about 90% of classical RTT cases (Amir et al., 1999;

Abbreviations: RTT, Rett syndrome; Mecp2, methyl–CpG–binding protein 2; CORT, corticosterone; PVN, paraventricular nucleus of the hypothalamus; Crh, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; GR, glucocorticoid receptors; MR, mineralocorticoid receptors; wt, wild-type; pnd, postnatal day; BDNF, Brain Derived Neurotrophic Factor; hz, hemizygous.

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Chahrour and Zoghbi, 2007). *MECP2* encodes a protein that specifically binds to methylated DNA and mainly acts as a key transcriptional regulator (Defossez and Stancheva, 2011; Guy et al., 2011).

The creation of RTT mouse models has provided a major breakthrough in understanding this severe neurodevelopmental disorder (Calfa et al., 2011; Ricceri et al., 2008). Shortly after the creation of Mecp2-null mice (Chen et al., 2001; Guy et al., 2011), a mouse which expresses a truncated form of *Mecp2* gene (Mecp2-308 mice) (Shahbazian et al., 2002), has been generated. In line with RTT patients carrying C-terminal deletions of the *MECP2* gene (about 10% of RTT cases) (Chahrour and Zoghbi, 2007), this RTT mouse model shows a later onset of symptoms and longer life expectation than null mutants (Ricceri et al., 2008). How mutations in the *MECP2* gene lead to the symptomatology and the characteristic neuropathological signs of RTT is however still unknown and no effective therapy is currently available for this disabling syndrome. Indeed, given the extreme complexity of RTT, even a partial restoration would represent a big step forward.

Anxiety is a typical feature of RTT and a key component of the disorder (Hagberg, 2002; Mount et al., 2002; Robertson et al., 2006; Sansom et al., 1993). Recent evidence in RTT mouse models however suggests that anxiety-like behaviours are strictly dependent on Mecp2 mutation types (for a review see (Chao and Zoghbi, 2012)). Consistently, many, but not all individuals with RTT show this symptom (Mount et al., 2002). These results highlight the importance of better understanding the phenotypic consequences of different pathogenic Mecp2 mutations.

Recently, disturbances in serotonergic neurotransmission in the hippocampus have been proposed to be linked to behavioural abnormalities of RTT, such as increased anxiety-like behaviours and reduced exploratory locomotion (Isoda et al., 2011).

Abnormal stress response, as evidenced by elevated serum corticosterone (CORT) levels and increased gene expression of the corticotropin-releasing hormone (Crh), was also found in Mecp2-*308* mice (McGill et al., 2006). Secreted glucocorticoids in the brain bind to glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) and exert a negative feedback which regulates the hypothalamic-pituitary-adrenal (HPA) activity and dampens the central drive of the axis (Dallman et al., 1994; de Kloet, 2003). Even though more recent studies question this view (Bradbury et al., 1991; Wellhoener et al., 2004), activation of MR is traditionally considered to be essential for maintenance of the basal circadian rhythm of glucocorticoids secretion, because of their high affinity and low capacity, whereas activation of GR is considered to be required for the stress response and the subsequent recovery of homeostasis via negative feedback (De Kloet and Reul, 1987; De Kloet et al., 1998). A key limbic regulator of the HPA axis is the hippocampus, which highly expresses both receptor types in rodents (De Kloet and Reul, 1987; De Kloet et al., 1998; Tasker and Herman, 2011). Mecp2-308 mice and wild-type (wt) mice respond similarly to treatment with the synthetic glucocorticoid dexamethasone, by suppressing endogenous CORT production and Crh transcription in the hypothalamus (McGill et al., 2006). This result suggests that the negative feedback arm of the HPA axis is functionally intact in Mecp2-308 mice.

Apart from their role in HPA regulation, ligand-activated GR and MR can contribute to the regulation of gene transcription and regulate a variety of cellular responses, including cellular structure and signal transduction (De Kloet et al., 1998). In the hippocampus, both MR and GR participate in corticosteroid-induced transcriptional repression of the *5-HT1a* receptor gene (Meijer et al., 2000; Ou et al., 2001). This neurochemical receptor population critically modulates anxiety-like behaviours (Akimova et al., 2009; Holmes et al., 2003; Kusserow et al., 2004) and is particularly involved in the reciprocal interaction between the brain serotonergic system and the HPA axis (Andrews and Matthews, 2004; Lanfumey et al., 2008).

At the behavioural level, hippocampal MR have been suggested to be involved in processes of situation evaluation and response selection (Meijer et al., 1998; Oitzl and de Kloet, 1992) and in the control of adequate patterns of reactivity towards environmental stimuli (Oitzl and de Kloet, 1992; Oitzl et al., 1994).

As a whole, these evidence suggest that modulation of MR and GR in the hippocampus of Mecp2-308 mice may be a strategy to contrast RTT-related abnormalities in neuroendocrine regulation and behavioural adaptation.

Perinatal environment can impact the developing brain (Lupien et al., 2009; Oitzl et al., 2011; Viltart and Vanbesien-Mailliot, 2007), causing long lasting structural and functional changes in the central nervous system (Spencer et al., 2011; Tremblay, 2011; Vieau, 2011). Glucocorticoid hormones play an important role in this context (for a review: (Catalani et al., 2011)): mother–offspring pituitary–adrenal inter-relationship is at play during postnatal life in rodents (Angelucci et al., 1983) and maternal transmission of circulating levels of CORT through lactation has been demonstrated to at least partially explain maternal influences on offspring phenotype at adulthood (Macri et al., 2009). Consistent with a dose-dependent effect of neonatal stress exposure (Coutellier et al., 2009; Macri and Wurbel, 2006; Macri et al., 2011), offspring of mothers whose drinking water was supplemented with moderate doses of CORT through lactation showed 'positive' effects or resilience. By contrast, elevated doses, comparable to those elicited by strong stressors, caused developmental disruption (Catalani et al., 2011).

Several lines of evidence in animal research point to the HPA axis as one key target of glucocorticoid programming early in development (Meaney et al., 2007). Moderate levels of neonatal CORT supplemented in the maternal drinking water in fact reduce adult stress reactivity, HPA-associated dysfunctions and anxiety-related behaviours. Moreover, alterations in early rearing conditions affect the levels of expression of the 5-HT1a receptor (Stamatakis et al., 2006; Vicentic et al., 2006) and Brain Derived Neurotrophic Factor (BDNF) (Lippmann et al., 2007; Macri et al., 2009) at adulthood. We therefore asked whether exposure to a low dose of CORT may affect early programming of the HPA axis in Mecp2-308 mice, thus contrasting RTT neurobehavioural features at adulthood.

To test this hypothesis, anxiety-like profile, reactivity to novelty, levels of locomotion and plasma CORT levels were evaluated in adult Mecp2-308 mice and wild-type (wt) littermates, neonatally exposed to a low dose of CORT (50 μg/ml, supplemented in the maternal drinking water from postnatal day (pnd) 1 to pnd 7) (see Fig. 1). At the end of behavioural testing, brains were dissected and hippocampal expression of *GR* and *MR* and *5-HT1a* receptor genes were also analyzed to determine whether alterations are evident in mutant mice and CORT treatment effects thereon. Since functional interactions between Mecp2 and BDNF have been reported (Chang et al., 2006; Zeev et al., 2009), and converging evidence point to abnormal BDNF signalling in association with the RTT phenotype in mouse models (Kondo et al., 2008), hippocampal expression of BDNF was also evaluated.

2. Materials and methods

2.1. Subjects

Breeding pairs of Mecp2-308 mice [heterozygous females and hemizygous (hz) males] were bred in our animal facility and obtained from crossings of Mecp2-308 mice from the Jackson Laboratories (USA) [B6.129S-Mecp2tm1Hzo/J, stock number: 005439; backcrossed to C57BL/6J mice for at least 12 generations]. Ten days