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BRAIN-DERIVED NEUROTROPHIC FACTOR HETEROZYGOUS MUTANT RATS SHOW SELECTIVE COGNITIVE CHANGES AND VULNERABILITY TO CHRONIC CORTICOSTERONE TREATMENT

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Abstract—Brain-derived neurotrophic factor (BDNF) is a widely expressed neurotrophin involved in neurodevelopment, neuroprotection and synaptic plasticity. It is also implicated in a range of psychiatric disorders such as schizophrenia, depression and post-traumatic stress disorder. Stress during adolescence/young adulthood can have long-term psychiatric and cognitive consequences, however it is unknown how altered BDNF signaling is involved in such effects. Here we investigated whether a congenital deficit in BDNF availability in rats increases vulnerability to the long-term effects of the stress hormone, corticosterone (CORT). Compared to wildtype (WT) littermates, BDNF heterozygous (HET) rats showed higher body weights and minor developmental changes, such as reduced relative brain and pituitary weight. These animals furthermore showed deficits in short-term spatial memory in the Y-maze and in prepulse inhibition and startle, but not in object-recognition memory. CORT treatment induced impairments in novel-object recognition memory in both genotypes but disrupted fear conditioning extinction learning in BDNF HET rats only. These results show selective behavioral changes in BDNF HET rats, at baseline or after chronic CORT treatment and add to our understanding of the role of BDNF and its interaction with stress. Importantly, this study demonstrates the utility of the BDNF HET rat in investigations into the pathophysiology of various psychiatric disorders.

Key words: brain-derived neurotrophic factor, BDNF heterozygous rat, schizophrenia, cognition.

INTRODUCTION

The effects of chronic stress or allosteric overload (McEwen, 2008) during the adolescent and young-adult phase of neurodevelopment is known to have long-term consequences which may manifest in adulthood as various psychiatric disorders (Kaufman et al., 2000). The vulnerability of the brain during this period is due to the ongoing and fragile process of brain modeling in response to a multitude of internal and external cues (Eiland and Romeo, 2013). The hypothalamic–pituitary axis (HPA) is the main response system to the effects of stress and numerous studies have shown that in adolescents the release of glucocorticoids such as corticosterone (CORT) by the adrenal glands is greater compared to adults, suggesting increased sensitivity to stress during this period of growth (McCormick and Mathews, 2007). CORT binds to mineralocorticoid (MR) or glucocorticoid receptors (GR) which are located in several brain regions including the prefrontal cortex (PFC), amygdala and hippocampus. The hippocampus in particular has a significant role in regulating basal tone of the HPA-axis and its response to stress (Vazquez et al., 1996). Upon CORT binding to MRs or GRs, a range of genomic and non-genomic responses are initiated, such as hippocampal feedback to the HPA, modulation of synaptic plasticity, and influencing the expression of various proteins such as brain-derived neurotrophic factor (BDNF) (Joels and Baram, 2009).

BDNF is expressed widely throughout the brain and plays a major role in neurodevelopment, and plasticity. It is initially synthesized as the larger precursor prepro-BDNF (30–35 kDa) which is proteolytically converted to proBDNF (28 kDa) and then to BDNF (Huang and Reichardt, 2001). The effects of BDNF are mediated predominantly by binding to the tropomyosin-related kinase B (TrkB) receptor. Neuronal release of proBDNF is a highest during the early stages of brain development and then reduces in adolescence and adulthood (Yang et al., 2009). Interestingly, we have shown that levels of BDNF rise and fall in a sex-dependent manner at these developmental time points (Hill et al., 2012).

We have previously reported on the long-term effects of chronic CORT treatment in male and female BDNF

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Abbreviations: ANOVA, analysis of variance; BDNF, Brain-derived neurotrophic factor; CORT, corticosterone; CS, conditioning stimulus; GR, glucocorticoid receptor; HET, heterozygous; HET CORT, HET rats receiving CORT; HPA, hypothalamic–pituitary axis; MR, mineralocorticoid receptor; NMDA, N-methyl-D-aspartate; PFC, prefrontal cortex; PTSD, posttraumatic stress disorder; TrkB, tropomyosin-related kinase B; US, unconditioned stimulus; WT, wildtype; WT Contr, WT rats receiving tap water.

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heterozygous (HET) mice, which have approximately 50% reductions of brain BDNF levels (Klug et al., 2012). In adulthood, male, but not female BDNF HET mice treated with CORT showed significant deficits in short-term spatial memory and markedly elevated levels of the NR2B subunit of the N-methyl-D-aspartate (NMDA) receptor in the dorsal hippocampus (Klug et al., 2012) which is involved in spatial memory (Fanselow and Dong, 2010). This previous work suggested that a deficiency in BDNF renders the brain more vulnerable to the effects of chronic stress during adolescence in a sex-specific manner (Wu et al., 2013) and that consequent alterations in glutamatergic neurotransmission could underlie some of the behavioral effects. However, similar studies have not been done in rats, even though this is the preferred species for the study of psychiatric disorders (Parker et al., 2014). Rats have a richer behavioral repertoire than mice and the similarities they have with human neurophysiology make them a more translationally relevant species. We therefore used BDNF HET rats, with the aim to phenotype the animals for a number of behavioral measures and to assess the effect of chronic young-adult CORT treatment.

We recently reported on the genotype and CORT treatment effects on anxiety and depression-like behavior (Gururajan et al., 2014). Here we present findings from tests of cognition and other behaviors. Because few studies have been done with this model, we also verified body weight and organ weights. Lastly, as we had previously done for BDNF HET mice we examined effects on BDNF, TrkB and NMDA receptor subunit level expression in the dorsal hippocampus.

EXPERIMENTAL PROCEDURES

Animals

All rats were obtained from a breeding colony at the institute which was set up with wildtype (WT) Sprague–Dawley and BDNF HET rats on a Sprague–Dawley genetic background (SD-BDNF^{tm1sage}) from SAGE® Labs. After weaning, male offspring were housed by genotype in individually ventilated cages in groups of three to four with free access to tap water and standard pellet food. They were kept in a temperature-controlled environment (22 °C) on a 12/12-h light/dark cycle (lights on 0700–1900 h). All procedures were conducted according to the guidelines in the Australian Code of Practise for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council of Australia, 2004) and approved by the Animal Ethics Committee of the Florey Institute of Neuroscience and Mental Health.

At the end of the behavioral experiments, the rats were killed by intraperitoneal injection of Euthal (300 mg/kg) and decapitation. The brain was rapidly removed, weighed and frozen at −80 °C. Whole-pituitary, heart, kidney, adrenal and seminal vesicle weights were also obtained to assess if there were any developmental alterations in the BDNF HET rats or as a result of CORT treatment. Organ weights were expressed as percentage of body weight.

CORT treatment

CORT treatment consisted of 21 days of 200 mg/L CORT hemisuccinate (4-pregnen-11β 21-DIOL-3 20-DIONE 21-hemisuccinate, Steraloids, Newport, RI, USA) in the drinking water, starting at 8 weeks of age (Gourley and Taylor, 2009). To achieve this, the pH of the solution was initially raised to 12–13 by adding 10 N NaOH and it was left to stir for several hours at 4 °C. Following dissolution of the CORT, the pH was brought back down to 7–7.4 using 10 N HCl. CORT bottles were wrapped in aluminum foil to protect against light-induced degradation. Every 3–4 days, the amount of fluid consumed was measured based on bottle weight, after which the bottles were replaced with fresh ones. Thus, there were four experimental groups: WT rats receiving tap water (WT Contr), WT rats receiving CORT solution (WT CORT), BDNF HET rats receiving tap water (HET Contr) and BDNF HET rats receiving CORT (HET CORT). After the 3-week treatment period ended, the animals received normal tap water for two further weeks until behavioral testing. To determine the short- and long-term effects of genotype and treatment on general health, food intake and metabolism, body weights were obtained at the beginning of treatment (week 8), at the end of treatment (week 11) and at the start of behavioral testing (week 13). Behavioral testing was done between 0900 and 1600 h with 2–4-day intervals with the tests presumed least stressful first and most stressful last. The number of animals used per group for each test is indicated in Table 1.

Behavioral testing

Y-maze. Short-term spatial memory was assessed using the Y-maze (Klug and van den Buuse, 2012). The maze consisted of three arms (50 × 16 × 31 cm) with visual cues at the end of each arm and a triangular section in the middle. The maze floor was covered with cage bedding which was mixed between trials to reduce olfactory cues. During the training session, the rats were placed facing the wall of one of the arms (start arm) and allowed to explore the maze for 10 min with one of the two other arms closed off (novel arm). After a 1-h retention period, the rats were placed back into the start arm and allowed to explore all three arms for 5 min. The location of start, novel and familiar arms was randomized between animals. Behavior was recorded on video and later analyzed using TopScan (Version 2.0, Cleversys,

Table 1. Number of animals per group in each experiment

	WT Contr	WT CORT	HET Contr	HET CORT
Organ weight	13	16	15	16
Y-maze	7	12	12	12
Novel-object recognition	10	12	11	12
Fear conditioning	13	16	12	15
PPI	11	11	15	15