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Long-term effects of combined neonatal and adolescent stress on brain-derived neurotrophic factor and dopamine receptor expression in the rat forebrain

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ABSTRACT

Altered brain-derived neurotrophic factor (BDNF) signalling and dopaminergic neurotransmission have been shown in the forebrain in schizophrenia. The 'two hit' hypothesis proposes that two major disruptions during development are involved in the pathophysiology of this illness. We therefore used a 'two hit' rat model of combined neonatal and young-adult stress to assess effects on BDNF signalling and dopamine receptor expression. Wistar rats were exposed to neonatal maternal separation (MS) stress and/or adolescent/young-adult corticosterone (CORT) treatment. At adulthood the medial prefrontal cortex (mPFC), caudate putamen (CPu) and nucleus accumbens (NAc) were analysed by qPCR and Western blot. The 'two hit' combination of MS and CORT treatment caused significant increases in BDNF mRNA and protein levels in the mPFC of male, but not female rats. BDNF mRNA expression was unchanged in the CPu but was significantly reduced by CORT in the NAc. DR3 and DR2 mRNA were significantly up-regulated in the mPFC of two-hit rats and a positive correlation was found between BDNF and DR3 expression in male, but not female rats. DR2 and DR3 expression were significantly increased following CORT treatment in the NAc and a significant negative correlation between BDNF and DR3 and DR2 mRNA levels was found. Our data demonstrate male-specific two-hit effects of developmental stress on BDNF and DR3 expression in the mPFC. Furthermore, following chronic adolescent CORT treatment, the relationship between BDNF and dopamine receptor expression was significantly altered in the NAc. These results elucidate the long-term effects of 'two hit' developmental stress on behaviour.

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1. Introduction

Schizophrenia is a severely debilitating neurodevelopmental disorder which affects approximately 1% of the population and is caused by a combination of genetic alterations and environmental factors. Meta-analysis studies have shown a significant association between blood levels of brain-derived neurotrophic factor (BDNF), as well as the Val66Met polymorphism in the BDNF gene, with schizophrenia [1,2]. In addition, human post-mortem studies have found significantly reduced mRNA and protein levels of BDNF and its receptor, tropomyosin-related kinase B (TrkB), in the prefrontal cortex of patients with schizophrenia [3,4]. Furthermore, this reduction in BDNF mRNA in the prefrontal cortex appears to be driven by changes in promoter II-IX of the BDNF gene [5].

BDNF has a complex genetic structure consisting of eight distinct promoters, each of which contains a 5′ untranslated exon (I–VIII) that is spliced to one 3′ protein coding exon (exon IX), in addition to a transcript containing a 5′ extended protein coding exon (IXa) [6]. These exon-specific promoters are region-specific and serve a number of functions: they can differentially regulate local BDNF expression within somatic and dendritic compartments [7]; they contain different elements, such as cAMP response element (CRE) for the CRE-binding protein (CREB) transcription factor and estrogen response elements (ERE), which allow for additional regulation of BDNF transcription [8–11], and they are differentially responsive to environmental stress [12–14]. Remarkably, all of the above mentioned exon-specific promoters transcribe a single precursor preproBDNF (32 kDa), which is then cleaved to a minor truncated protein (proBDNF, 28 kDa) and mature BDNF (mBDNF, 13.5 kDa) [15]. The mature BDNF isoform exerts the majority of its effects through TrkB, triggering a number of intracellular signalling

Abbreviations: BDNF, brain-derived neurotrophic factor; DR2, dopamine receptor 2; DR3, dopamine receptor 3; MPFC, medial prefrontal cortex; CPu, caudate putamen; NAc, nucleus accumbens; CORT, corticosterone; MS, maternal separation

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pathways, including MAP kinase or ERK kinase-mitogen-activated protein kinase (MEK-MAPK), phosphatidylinositol-3-kinase (PI-3-K), and phospholipase C-γ (PLC-γ) [16].

Excessive dopaminergic neurotransmission has been associated with the psychotic symptoms of schizophrenia and dopamine receptor (DR) antagonism is the core mechanism of action of most antipsychotic drugs [17–19]. BDNF released from dopamine neurons is responsible for the induction of DR3 expression in the nucleus accumbens in development and adulthood [20]. Conversely, DR1- and DR2-mediated activation of calcium signalling cascades has been shown to increase striatal BDNF expression [21] and chronic treatment with antipsychotic drugs has been shown to reduce BDNF expression in rats [22,23]. Hence, a strong relationship appears to exist between BDNF and DA signalling.

The 'two hit' hypothesis suggests that schizophrenia is caused by a combination of disruptions during specific periods of development. The first 'hit' occurs during the perinatal period and causes increased vulnerability of the brain to a second 'hit' during the adolescent/young-adult period, which may then trigger disease onset [24]. However, the effect of such 'hits' on BDNF signalling and dopamine neurotransmission remains unclear. Previous studies have shown that neonatal maternal separation (MS) in rats induced disruptions in prepulse inhibition [25,26] and long-term cognitive impairments [27]. BDNF expression following MS appears to be time-dependent, with the immediate effects being an up-regulation of BDNF mRNA in hippocampal and cortical regions [27,28], while long-term effects of MS lead to a reduction in hippocampal BDNF mRNA expression [27]. In addition, chronic treatment with the stress hormone, corticosterone (CORT), has been shown to induce disruptions to cortical and prefrontal BDNF mRNA and protein [29,30]. Local infusion of CORT in the prefrontal cortex increases dopamine efflux [31], while chronic (2 weeks) CORT treatment causes a reduction in DR2 receptor mRNA expression in the dorsal striatum of rats [32]. We have modelled the 'two hit' scenario in rats by a combination of MS and chronic treatment with CORT. Our previous studies demonstrated deficits in spatial memory, reduced mRNA expression of BDNF in the hippocampus [33], and an inhibitory effect of the two 'hits' on dopaminergic regulation of prepulse inhibition [34]. The observations of a spatial memory deficit were recently confirmed and expanded in a separate cohort, whereby we found that the Y-maze deficit in 'two hit' rats was specific to males, and further, that female, but not male 'two hit' rats, displayed an anhedonic phenotype [35]. These behavioural phenotypes were accompanied by male-specific and female-specific reductions in mature BDNF protein expression in the dorsal and ventral hippocampus, respectively [35], thus suggesting that MS and chronic adolescent CORT can cause long-term effects on BDNF expression and that this may regulate adult behaviour. However, it is unclear how these stressors impact on forebrain regions implicated in schizophrenia, such as the prefrontal cortex and striatum.

Previous studies have shown reduced mRNA expression of BDNF and its receptor TrkB in the prefrontal cortex of schizophrenia patients [36]. In addition, significant alterations in the mRNA expression of DR1 and DR2 have been shown in the dorsolateral prefrontal cortex of schizophrenia patients compared to controls [37]. Alterations in striatal DR2 density remain a consistent finding in schizophrenia [38]. Furthermore, alterations in D2/D3 mRNA expression have been shown in the nucleus accumbens of a methylazoxymethanol acetate (MAM)-treated rat model of schizophrenia [39], and antipsychotics such as haloperidol and clozapine alter DR2 and DR3 expression in the NAc of a genetic rat model of schizophrenia [40].

In the current study we sought to determine the long-term effects of MS and chronic CORT treatment, either individually or combined, on BDNF expression in discrete regions of the rat forebrain, including the medial prefrontal cortex (MPFC), caudate putamen (CPu) and nucleus accumbens (NAc). In order to gain a complete understanding of BDNF expression and signalling, we measured exon-specific BDNF mRNA as well as mature BDNF protein levels. Furthermore, we analysed expression of dopamine DR2 and DR3 receptors to determine whether neurodevelopmental stressors at two different time points might impact on long term dopamine neurotransmission.

2. Methods

1. Animals and treatment paradigms

We used outbred Wistar rats which were obtained from Monash University (Clayton, VIC, Australia). Pregnant female rats were individually housed and checked twice daily for newborn litters. Within 24 h after birth, litters were culled to a maximum of eight pups, with equal sex ratio where possible. From postnatal day (pnd) 2 until pnd 14, pups were separated for a period of 3 h every day, from 09:00 to 12:00. While the dam was removed from the pups and placed into a new clean cage, the litter remained in the home cage but was transferred to a different room where they were out of sight, smell and hearing distance of the mother. The cage with pups was placed on a heat pad (approximately 30 °C) to maintain body temperature. Control, non-separated (NS) rats underwent 10–15 s of brief separation.

CORT (Sigma-Aldrich, St. Louis, MI, USA) treatment was delivered in the drinking water for 3 weeks from 8, 9 and 10 weeks of age. To this end, CORT was dissolved in a minimal amount of 100% ethanol and diluted to 50 mg/L with tap water (end concentration 0.5–1.0% ethanol). Control rats received the vehicle (veh) solution (0.5% ethanol in tap water).

Using only up to 2 pups/sex/treatment group from each litter, there were four treatment groups for each sex: NS/veh (males $n = 9$; females $n = 11$), MS/veh (males $n = 11$; females $n = 13$), NS/CORT (males $n = 11$; females $n = 11$) and MS/CORT (males $n = 12$; females $n = 11$). Animals were sacrificed at 16 weeks of age (6 weeks after finishing CORT treatment). Five - seven animals per group/sex were used for Western blot analysis, while 5–6 animals per group/sex were used for mRNA analysis. All procedures and experiments were approved by the Animal Experimentation Ethics Committee of the Florey Institute for Neuroscience and Mental Health, University of Melbourne.

2. Dissection of brains

Using razor blades and a stainless steel rat brain slice matrix with 1.0 mm coronal slice intervals, two 2 mm thick coronal slices were obtained (coronal section 1 (Fig. 1A): approximately bregma 4.70–2.70, coronal section 2 (Fig. 1B): approximately bregma 2.70–0.70) [41]. Slices were immediately removed from the matrix and submerged in the RNA stabilisation solution, RNA*later* (Life Technologies, USA). Using a scalpel and fine curved forceps, three areas of interest were dissected from the slices: the MPFC was dissected from coronal section 1 (Fig. 1A), whereas the CPu and NAc (ventral striatum) were dissected from coronal section 2 (Fig. 1B). The MPFC contained the prelimbic (PrL), infralimbic (IL), cingulate (Cg1) and secondary motor cortex (M2). The NAc contained the accumbens shell (AcbSh), the lateral accumbens shell (LAcbSh) and the accumbens core (AcbC) [41]. Care was taken not to include cortical tissue in the CPu sample.

3. Protein extraction, Western blot, RNA extraction and qPCR

Details of protein extraction and Western blot are provided in [35]. Reagents included radio-immunoprecipitation assay (RIPA) lysis buffer containing 150 mM sodium chloride (ChemSupply, AU), 1.0% Triton-X-100 (Sigma Aldrich), 0.1% sodium dodecyl sulphate (Sigma Aldrich) and 50 mM Tris pH 8.0 (Trizma Base, Sigma Aldrich), as well as protease inhibitor cocktail set III (1:200, Merck, Kilsyth, VIC, Australia) and set IV (1:50, Merck). Protein concentrations were determined using the bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, Rockford, IL, USA).

For Western blot analysis [35], 50 μg of protein was added with loading buffer (0.4 M Tris pH 6.8, 37.5% glycerol, 10% SDS, 1% 2

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