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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

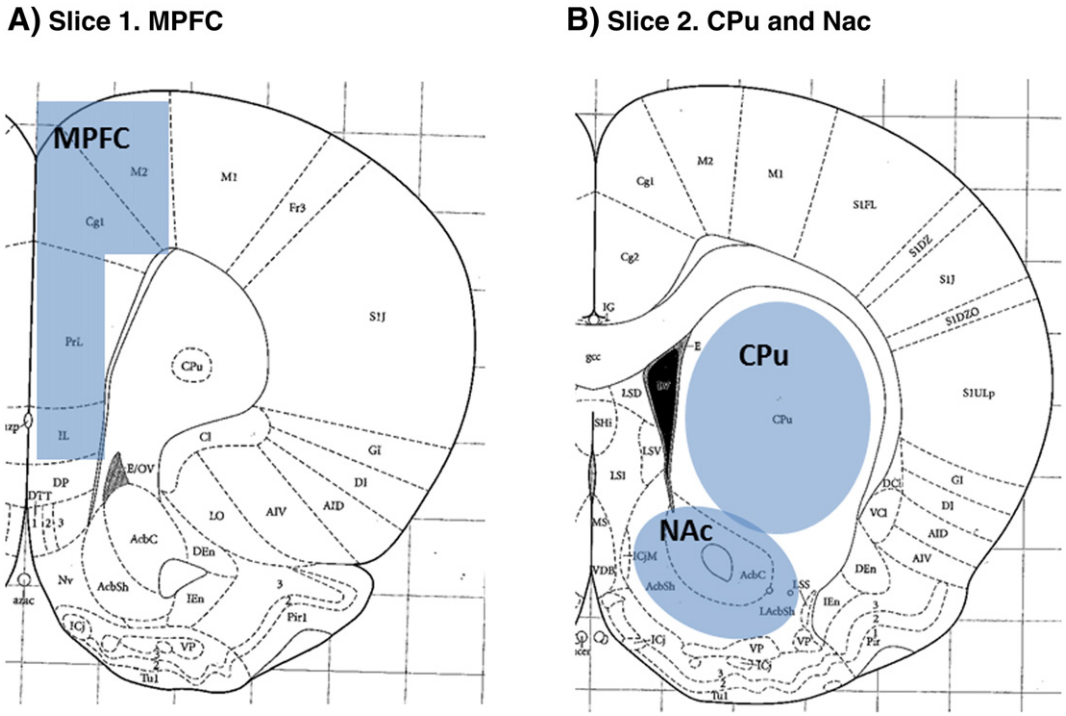

Fig. 1. Representative coronal sections of 2 mm slices taken from the forebrain, adapted from Paxinos and Watson 2005. (A) The mPFC (shaded in), which contained the prelimbic (PrL), infralimbic (IL), cingulate (Cg1) and secondary motor cortex (M2), was dissected from slice 1, approximately bregma 4.70–2.70. (B) The CPu and NAc (shaded in), which contained the accumbens shell (AcbSh), the lateral accumbens shell (LAcbSh) and the accumbens core (AcbC), were dissected from slice 2, approximately bregma 2.70–0.70.

mercaptoethanol, 0.5% bromphenol blue, dH_2O). Samples were denatured (10 min) at 95 °C before SDS-PAGE (15% or 10% acrylamide gel, 120 V, 1.5 h). After transfer to a nitrocellulose membrane, primary antibody incubation was done overnight at 4 °C. Primary antibodies were rabbit anti-BDNF (H-117, 1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-proBDNF (ab108383, 1:2000, Abcam, Cambridge, UK), rabbit anti-Phospho TrkB (1:1000, Signalway, College Park, MD, USA), rabbit anti-TrkB (1:1000, Santa Cruz Biotechnology) and mouse anti-β-actin (42 kDa; 1:10000, Sigma-Aldrich). The next day, anti-rabbit or anti-mouse IgG HRP-linked secondary antibodies (Cell Signalling, Danvers, MA, USA) were applied and images were captured using a Luminescence Image Analyzer (Fuji film LAS-4000, FujiFilm Life Science, Stamford, CT, USA). Multi Gauge software (FujiFilm Life Science) was used to quantify data for protein levels which were normalised against levels of β-actin and expressed relative to the average value of the male control group (NS/veh).

As described in detail previously [35], RNA was extracted using Trizol (Sigma Aldrich). RNA was incubated with DNase (1 μl/10 mg RNA, Roche, Indianapolis, IN, USA) for 3 h at 37 °C and purified with an RNeasy Mini Kit (Qiagen, Germantown, MD, USA). cDNA was prepared from RNA using Taqman Reverse Transcription reagents (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Primers were designed using Primer Express 3.0 (Applied Biosystems) (see [35]). For qPCR analysis, an EpMotion liquid handling robot (Eppendorf, North Ryde, NSW, Australia) and MicroAmp 384-well plates were used, with added SYBR Green Mastermix (Applied Biosystems). The 384-well plates were run on a Viia 7 real-time PCR system (Applied Biosystems) and gene expression was compared with 18 s according to the formula RT = $2^{-\Delta\Delta Ct}$ [42]. Data are expressed relative to the average value of the male control group (NS/veh).

4. Statistical analysis

ANOVA was used to determine statistically relevant changes between treatment groups for each sex, brain region and exon separately, the factors being MS, CORT treatment and sex (SYSTAT 13, Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant.

3. Results

1. BDNF, TrkB and dopamine receptor expression in the mPFC

ANOVA revealed a significant MS × CORT × sex interaction ($F(1,38) = 9.6$, $P = 0.004$) in the expression of protein-coding BDNF exon IX mRNA in the mPFC with male 'two hit' rats showing significantly increased expression compared to all other groups (Fig. 2A). In addition, a significant sex × MS effect was found for proBDNF protein expression ($F(1,42) = 8.8$, $P = 0.005$) with the male two-hit group showing the highest expression levels (Fig. 2B). A similar MS × CORT × sex interactive effect was found for mature BDNF protein levels ($F(1,45) = 5.1$, $P = 0.028$) with two-hit males showing increased levels compared to all other groups (Fig. 2C). The selective 'two hit' up-regulation after combined MS and CORT was not observed for any of the other, non-coding exons (Table 1). Instead, there were several other effects including markedly increased expression of BDNF exon I mRNA after MS ($F(1,37) = 22.8$, $P < 0.001$) but decreased expression of exon VII and VIII in males only ($F(1,38) = 13.7$, $P = 0.005$) (Table 1).

No significant changes were found in full-length TrkB protein expression (Fig. 2D); however, females showed significantly lower phosphorylated TrkB protein expression than males (main effect of sex: $F(1,40) = 5.55$, $P = 0.023$) (Fig. 2E).

A significant effect of MS ($F(1,32) = 6.3$, $P = 0.017$) and a significant MS × CORT × sex interaction ($F(1,32) = 5.3$, $P = 0.028$) were found for DR3 mRNA expression in the MPFC, with male 'two hit' rats showing significantly increased expression compared to all other groups (Fig. 2F). A significant effect of CORT ($F(1,35) = 7.5$, $P = 0.01$), MS ($F(1,35) = 7.9$, $P = 0.008$) and sex ($F(1,35) = 10.9$, $P = 0.002$) was found on DR2 mRNA expression in the MPFC (Fig. 2G). In addition, a significant CORT × sex interaction was found ($F(1,35) = 0.11$, $P = 0.04$). We then analysed males and females separately and found that CORT

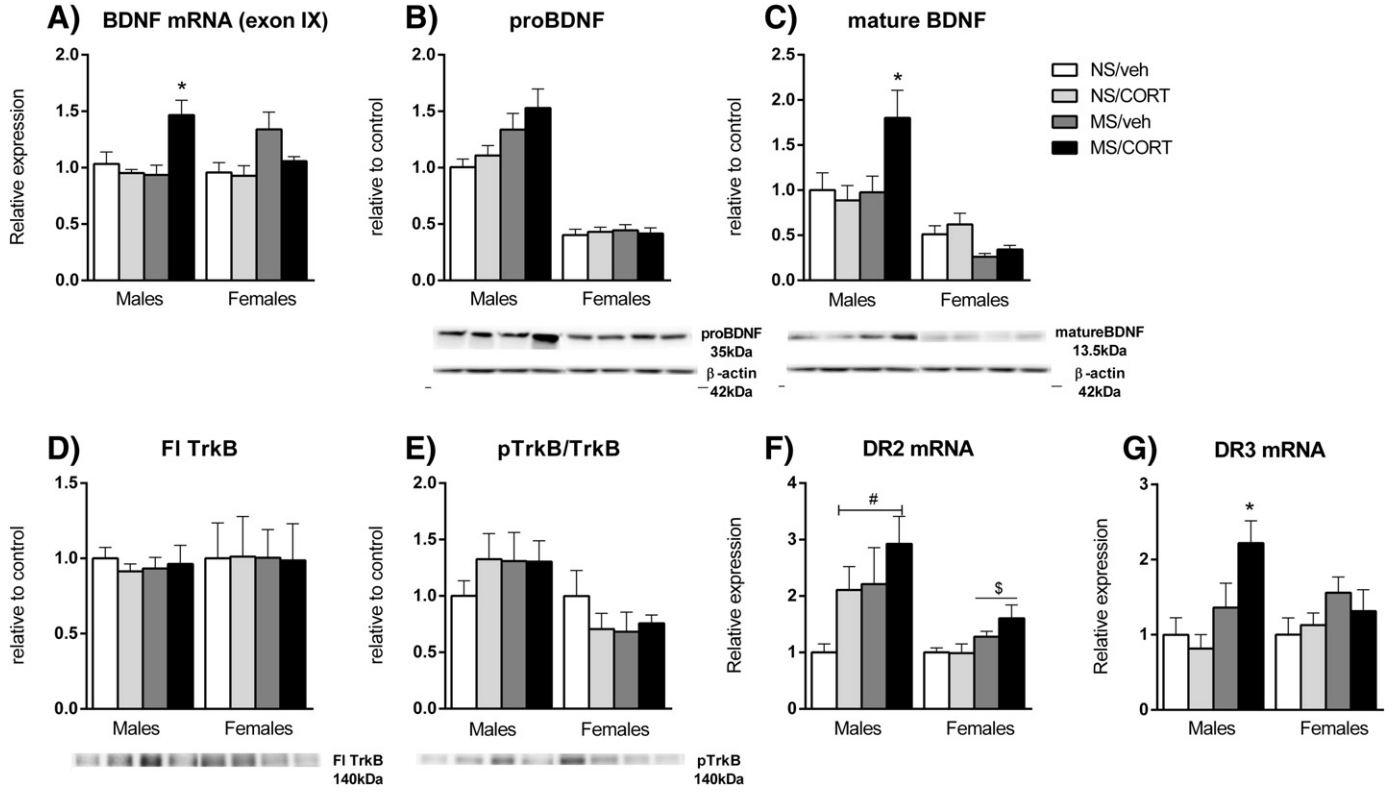

Fig. 2. BDNF-TrkB expression and signalling and dopamine receptor expression in the mPFC. (A) BDNF mRNA levels, (B) ProBDNF protein levels, (C) Mature BDNF protein levels, (D) Full-length TrkB protein expression, (E) Phosphorylated TrkB / full-length TrkB protein levels, (F) DR3 mRNA levels and (G) DR2 mRNA levels. ND/veh = non-deprived/vehicle-treated, ND/CORT = non-deprived/corticosterone-treated, MS/veh = maternally-deprived/vehicle-treated and MS/CORT = maternally-deprived/corticosterone-treated. * = significant MS × CORT × sex interaction $P < 0.05$, # over a horizontal line with vertical bars at either end = significant effects of CORT treatment, $ over a horizontal line = significant effects of MS, */#/$ = $P < 0.05$. $n = 5$–6/group.

significantly increased DR2 mRNA expression in males ($F(1,15) = 5.2$, $P = 0.03$), but not females, whereas MS significantly increased DR2 expression in females ($F(1,20) = 7.9$, $P = 0.01$), but not males.

The expression pattern of DR3 mRNA was similar to that of BDNF mRNA expression and linear regression analysis revealed a significant positive correlation between DR3 and BDNF mRNA expression (Fig. 3A; $r^2 = 0.33$, $P < 0.001$) in the mPFC. In contrast, DR2 expression did not correlate significantly with BDNF mRNA expression (Fig. 3B). However, DR2 expression did correlate significantly with both proBDNF (Fig. 3D; $P = 0.0003$, $r^2 = 0.28$) and mature BDNF (Fig. 3F; $P = 0.001$, $r^2 = 0.24$) protein expression, while no correlations were found between BDNF protein expression and DR3 (Fig. 3C and D).

2. BDNF, TrkB and dopamine receptor expression in the caudate putamen

Expression levels of BDNF in the CPu were low and variable (Fig. 4). A significant sex × CORT interaction was found in the mRNA expression of BDNF exons I ($F(1,38) = 4.3$, $P = 0.04$), II ($F(1,38) = 4.1$, $P = 0.04$) and IXa ($F(1,39) = 4.1$, $P = 0.04$), with females, but not males, showing an up-regulation following CORT treatment (Table 2). In addition, a significant MS × CORT interaction was found for exon I ($F(1,38) = 7.6$, $P = 0.009$) (Table 2). However, despite these exon-specific changes, total protein coding exon IX levels were unchanged in the CPu in all groups analysed (Fig. 4A). Females showed higher proBDNF ($F(1,48) = 18.7$, $P < 0.001$) and mature BDNF ($F(1,45) = 15.1$, $P < 0.001$) protein

Table 1
Exon-specific BDNF transcript expression in the medial prefrontal cortex of male and female rats. Data represent mean percentage fold-change from the non-separated/vehicle-treated male control group ± SEM.

Fold-change from male controls								
Transcript	Males			Females				
	CORT	MS	MS+CORT	Control	CORT	MS	MS+CORT	Effect
BDNF exon I	0.85 ± 0.13	1.46 ± 0.30	1.48 ± 0.21	1.20 ± 0.15	1.15 ± 0.23	1.86 ± 0.27	1.70 ± 0.13	↑MS, $P < 0.0001$
BDNF exon II	0.72 ± 0.06	0.92 ± 0.16	0.94 ± 0.11	0.91 ± 0.06	0.82 ± 0.09	1.18 ± 0.14	1.03 ± 0.05	↑MS, $P = 0.049$
BDNF exon III	0.94 ± 0.09	0.92 ± 0.09	0.78 ± 0.06	0.77 ± 0.33	0.70 ± 0.06	1.03 ± 0.06	0.79 ± 0.04	↓CORT, $P = 0.018$; MS × sex, $P = 0.007$
BDNF exon IV	0.92 ± 0.12	0.93 ± 0.15	1.05 ± 0.12	0.73 ± 0.04	0.77 ± 0.08	1.10 ± 0.09	0.91 ± 0.06	0
BDNF exon VI	0.83 ± 0.14	0.87 ± 0.15	0.96 ± 0.11	0.72 ± 0.07	0.62 ± 0.09	1.04 ± 0.15	0.78 ± 0.07	0
BDNF exon VII	0.99 ± 0.16	0.54 ± 0.14	0.65 ± 0.08	0.80 ± 0.11	0.57 ± 0.08	0.95 ± 0.16	0.60 ± 0.06	MS × sex, $P = 0.005$
BDNF exon VIII	0.85 ± 0.09	0.44 ± 0.10	0.68 ± 0.05	0.64 ± 0.40	0.52 ± 0.10	0.80 ± 0.11	0.54 ± 0.02	↓MS, $P = 0.02$; MS × sex, $P = 0.001$; MS × CORT × sex, $P = 0.036$
BDNF exon IXa	1.22 ± 0.14	0.76 ± 0.22	0.84 ± 0.04	0.81 ± 0.13	0.67 ± 0.15	1.0 ± 0.16	0.73 ± 0.02	MS × sex, $P = 0.031$
BDNF exon IX	0.95 ± 0.03	0.93 ± 0.08	1.46 ± 0.13	0.95 ± 0.08	0.92 ± 0.09	1.33 ± 0.15	1.06 ± 0.04	↑MS, $P = 0.002$; CORT × sex, $P = 0.009$; MS × CORT × sex, $P = 0.004$

MS = maternal separation, CORT = corticosterone treatment; ↑ indicates main effect as analysed by ANOVA.

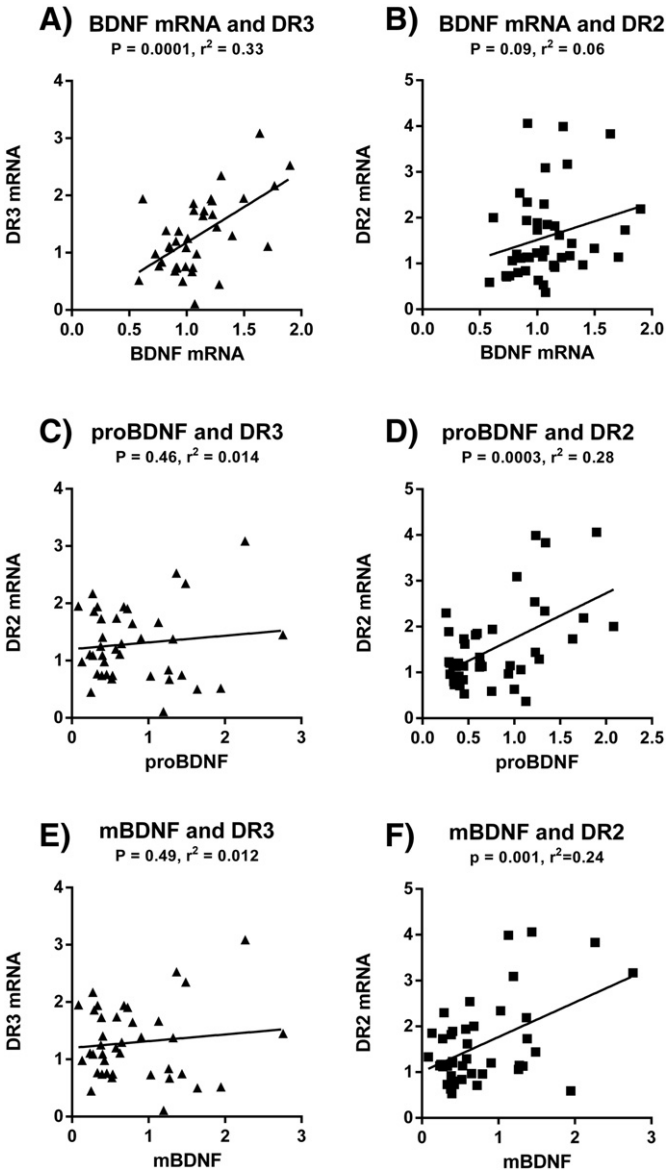

Fig. 3. Linear regression graphs showing correlation analysis of (A) BDNF mRNA (*x* axis) and DR3 mRNA (*y* axis) in the mPFC, (B) BDNF mRNA (*x* axis) and DR2 mRNA (*y* axis) levels in the mPFc, (C) proBDNF protein (x axis) and DR3 mRNA (y axis) levels in the MPFC, (D) proBDNF protein (x axis) and DR2 mRNA (y axis) levels in the MPFC, (E) mBDNF protein (x axis) and DR3 mRNA (y axis) levels in the MPFC, (F) mBDNF protein (x axis) and DR2 mRNA (y axis) levels in the MPFC.

expression than males; however, no further group differences were found (Fig. 4B and C).

Analysis of TrkB protein levels in the CPu revealed a significant MS × CORT interaction ($F(1,48) = 7.6$, $P = 0.008$) and a significant effect of sex ($F(1,48) = 4.12$, $P = 0.048$) with male 'two hit' rats showing a significant increase in full-length TrkB protein expression (Fig. 4D). However, a significant effect of sex ($F(1,45) = 9.1$, $P = 0.004$), an MS × CORT interaction ($F(1,45) = 5.1$, $P = 0.03$) and a sex × CORT interaction ($F(1,45) = 6.2$, $P = 0.016$) reflected that TrkB phosphorylation (pTrkB/TrkB) was reduced in male 'two hit' rats only (Fig. 4E).

Male rats showed higher expression than females of DR3 ($F(1,36) = 14.5$, $P = 0.001$) and DR2 ($F(1,39) = 6.3$, $P = 0.015$) mRNA in the CPu; however, no further group differences were found (Fig. 4F and G). No significant correlations were found between BDNF mRNA and DR2 or DR3 mRNA expression, BDNF protein and DR2 or DR3 mRNA, or TrkB expression or phosphorylation and DR2 or DR3 mRNA expression in the CPu (data not shown).

3. BDNF, TrkB, and dopamine receptor expression in the NAc

Total, protein coding BDNF exon IX expression was significantly reduced following CORT treatment in the NAc (Fig. 5A; $F(1,36) = 6.0$, $P = 0.019$) and, in addition, females showed higher expression levels than males ($F(1,36) = 4.1$, $P = 0.049$). These effects of CORT and sex on exon IX expression appeared to be driven by similar alterations in exon III (main effect of CORT: $F(1,37) = 4.3$, $P = 0.045$; main effect of sex: $F(1,37) = 6.7$, $P = 0.013$), exon IV (CORT: $F(1,35) = 4.4$, $P = 0.043$; sex: $F(1,35) = 4.8$, $P = 0.035$) and exon IXa (sex: $F(1,36) = 4.6$, $P = 0.038$) (Table 3). A significant effect of sex ($F(1,45) = 29.5$, $P < 0.001$) was found for proBDNF protein expression with females showing higher expression overall; however, no further group differences were found here (Fig. 5B). A trend for a CORT × sex interaction was found for mature BDNF protein expression (Fig. 5C; $F(1,42) = 3.8$, $P = 0.056$) with males, but not females showing reduced expression following CORT treatment.

With respect to TrkB protein expression, a significant effect of sex ($F(1,47) = 74.1$, $P < 0.001$) and a significant effect of MS ($F(1,47) = 8.93$, $P = 0.004$) were found, with MS causing an increase and females showing higher levels overall (Fig. 5D). A significant effect of sex ($F(1,47) = 49.8$, $P < 0.001$) and a significant effect of CORT ($F(1,47) = 8.98$, $P = 0.004$) were found on phosphorylated TrkB (pTrkB/TrkB) expression with reduced expression following CORT treatment and lower overall expression levels found in females (Fig. 5E).

Analysis of DR3 mRNA expression in the NAc showed a significant effect of CORT ($F(1,39) = 4.5$, $P = 0.04$), sex ($F(1,39) = 14.7$, $P < 0.001$) and a CORT × sex interaction ($F(1,39) = 4.7$, $P = 0.037$), with males but not females showing a marked and significant increase following CORT treatment (Fig. 5F). No significant effect of treatment or sex was found on DR2 receptor mRNA expression (Fig. 5G). Correlation analysis showed a significant negative correlation between BDNF exon IX mRNA expression and DR3 mRNA expression (Fig. 6A, $r^2 = 0.23$, $P = 0.002$) and DR2 mRNA expression (Fig. 6B, $r^2 = 0.29$, $P = 0.0002$) in the NAc. In addition, a modest, but significant negative correlation was also found between phosphorylated TrkB protein expression and DR3 mRNA expression in this brain region in males only (Fig. 6C, $r^2 = 0.29$, $P = 0.01$).

4. Discussion

We examined the long-term effects of early postnatal stress in the form of MS, adolescent/young-adult stress in the form of chronic CORT treatment, and the combined effect of the two stressors on BDNF signalling as well as DR2 and DR3 mRNA expression in discrete forebrain regions. In the MPFC of adult male rats, significant increases in BDNF mRNA and protein levels, as well as DR3 expression were observed following the 'two hit' combination of neonatal and adolescent/young-adult stress. This was not observed in female rats. Male-specific, 'two hit' effects were also evident in the CPu, with alterations in TrkB protein expression and phosphorylation that may reflect a disruption in the anterograde transport of BDNF from the MPFC to the CPu. BDNF mRNA expression was significantly reduced by CORT treatment in the NAc; however, both DR3 and DR2 were significantly up-regulated following CORT treatment and a negative correlation between BDNF mRNA and DR3 and DR2 mRNA was found in the NAc.

In addition to region- and sex-specific effects of MS and CORT on BDNF exon IX expression and protein levels, we found several BDNF exon-specific effects. For example, in the mPFC, MS significantly increased expression of exons I and II. Thus, effects on BDNF exon IX expression and protein levels were associated with a number of differential exon-specific changes depending on the region investigated or sex of the animals. This may represent alternate mechanisms which

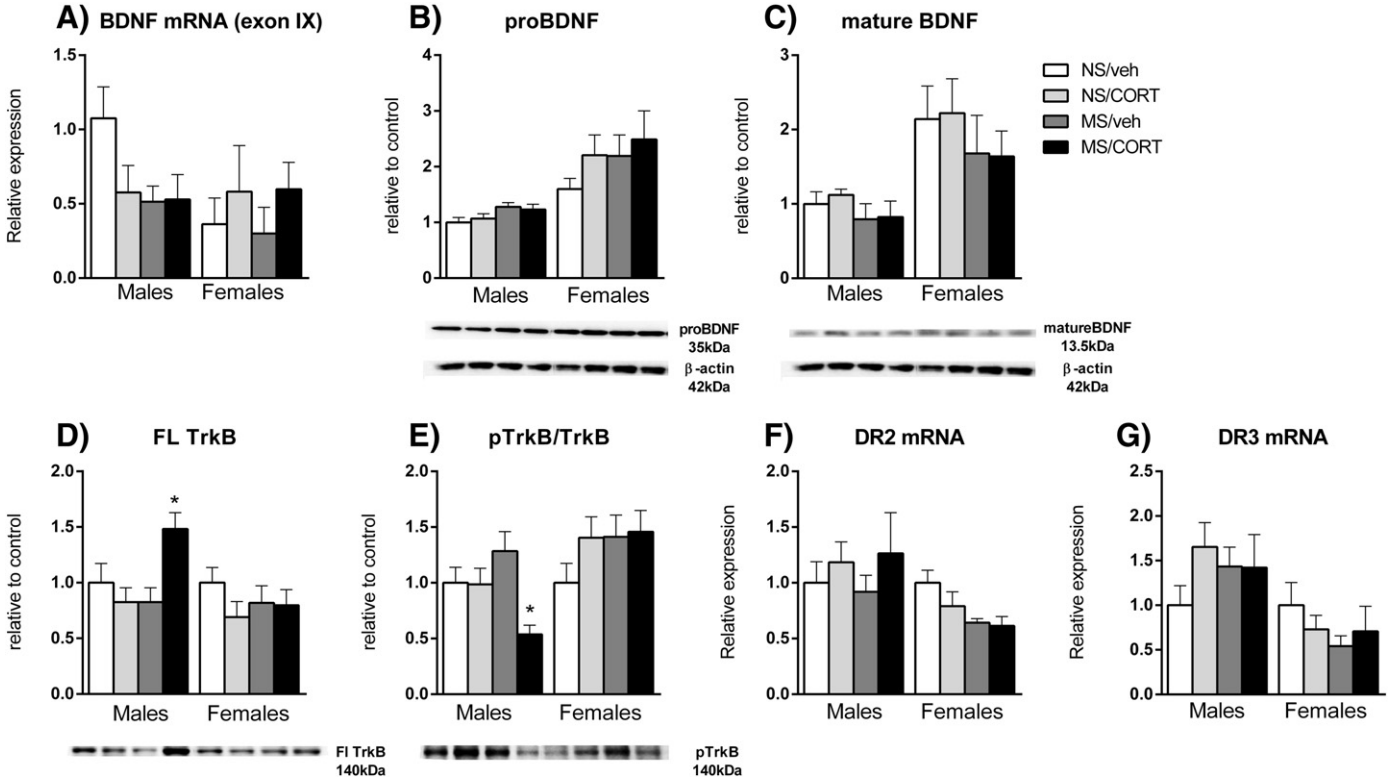

Fig. 4. BDNF-TrkB expression and signalling and dopamine receptor expression in the CPu. (A) BDNF mRNA levels, (B) Pro BDNF protein levels, (C) Mature BDNF protein levels, (D) Full-length TrkB protein levels, (E) Phosphorylated TrkB / full-length TrkB protein levels, (F) DR3 mRNA levels, (G) DR2 mRNA levels. ND/veh = non-deprived/vehicle-treated, ND/CORT =non-deprived/corticosterone-treated, MS/veh = maternally-deprived/vehicle-treated and MS/CORT = maternally-deprived/corticosterone-treated. n = 5–6/group. * = significant MS × CORT × sex interaction $P < 0.05$.

drive total BDNF exon IX expression between brain regions and between the sexes. In contrast to previous studies [43], protein levels of BDNF in the current study were dissimilar to exon IV expression, but did parallel expression of protein-coding exon IX expression.

There were also several effects which were unique to MS or to CORT treatment, without 'two hit' interactions. Previous reports on the immediate effects of MS stress in the form of motherless rearing showed a decrease in prefrontal cortex BDNF protein expression [44], while adult stress in the form of 2 days of forced-swim stress caused an immediate increase in BDNF protein levels in the ventral OFC of adult rats [45]. Chronic stress in the adult rat has been shown previously to cause an immediate increase in BDNF mRNA levels in the prefrontal cortex [46,47]. Furthermore, 3 weeks of CORT treatment in rats causes an immediate decrease specifically in exons II and IV transcripts in the frontal cortex and hippocampus [14]. Overall, it would appear that the timing of the stressors is an important factor in the resulting changes in BDNF expression. For example, total BDNF mRNA levels measured 0, 2, 24 and 72 h after foot shock stress was increased in the prelimbic and infralimbic mPFC at 0 and 2 h post-stress, but unchanged at 24 or 72 h [48]. In addition, in the human prefrontal cortex exon-specific expression of BDNF has been shown to change significantly throughout development, with protein expression following a similar pattern to exon IV expression [43]. This may suggest that alternative promoters are used to drive BDNF expression in a time-dependent manner, and therefore applying a particular stressor at a specific period of development will have differential effects on the various promoter-specific exons.

Different types of stress paradigms applied may also elicit differential effects on BDNF expression. For example, chronic restraint stress during adulthood led to an immediate decrease, while repeated injections of the corticotrophin-releasing factor receptor agonist, urocortin, to the basolateral amygdala in adulthood actually increased BDNF protein expression in the prefrontal cortex [49]. Furthermore, predator

Table 2
Exon-specific BDNF transcript expression in the dorsal caudate nucleus/putamen of male and female rats. Data represent mean percentage fold-change from the non-separated/vehicle-treated male control group ± SEM.

Fold-change from male controls								
Transcript	Males			Females				
	CORT	MS	MS+CORT	Control	CORT	MS	MS+CORT	Effect
BDNF exon I	0.29 ± 0.12	0.26 ± .11	0.48 ± 0.16	0.42 ± 0.18	0.47 ± 0.21	0.27 ± 0.13	0.70 ± 0.21	↑MS × CORT $P = 0.009$, sex × CORT, $P = 0.04$
BDNF exon II	0.41 ± 0.16	0.31 ± 0.13	0.44 ± 0.15	0.38 ± 0.16	0.53 ± 0.23	0.25 ± 0.13	0.55 ± 0.15	CORT × sex, $P = 0.049$
BDNF exon III	0.43 ± 0.18	0.38 ± 0.17	0.48 ± 0.19	0.33 ± 0.16	0.57 ± 0.29	0.26 ± 0.15	0.58 ± 0.16	0
BDNF exon IV	0.50 ± 0.21	0.32 ± 0.15	0.54 ± 0.20	0.35 ± 0.16	0.71 ± 0.47	0.28 ± 0.17	0.62 ± 0.18	0
BDNF exon VI	0.41 ± 0.18	0.31 ± 0.15	0.43 ± 0.16	0.34 ± 0.14	0.64 ± 0.10	0.24 ± 0.15	0.47 ± 0.13	0
BDNF exon VII	0.55 ± 0.28	0.73 ± 0.32	0.47 ± 0.32	0.31 ± 0.14	0.55 ± 0.29	0.25 ± 0.12	0.43 ± 0.13	0
BDNF exon VIII	0.67 ± 0.35	0.69 ± 0.32	0.49 ± 0.20	0.35 ± 0.16	0.67 ± 0.34	0.21 ± 0.11	0.39 ± 0.12	0
BDNF exon IXa	0.61 ± 0.29	0.74 ± 0.34	0.42 ± 0.17	0.25 ± 0.11	0.66 ± 0.34	0.22 ± 0.12	0.38 ± 0.10	CORT × sex, $P = 0.049$
BDNF exon IX	0.40 ± 0.16	0.39 ± 0.13	0.44 ± 0.16	0.36 ± 0.17	0.58 ± 0.30	0.30 ± 0.17	0.59 ± 0.18	0

MS = maternal separation, CORT = corticosterone treatment; ↑ indicates main effect as analysed by ANOVA.

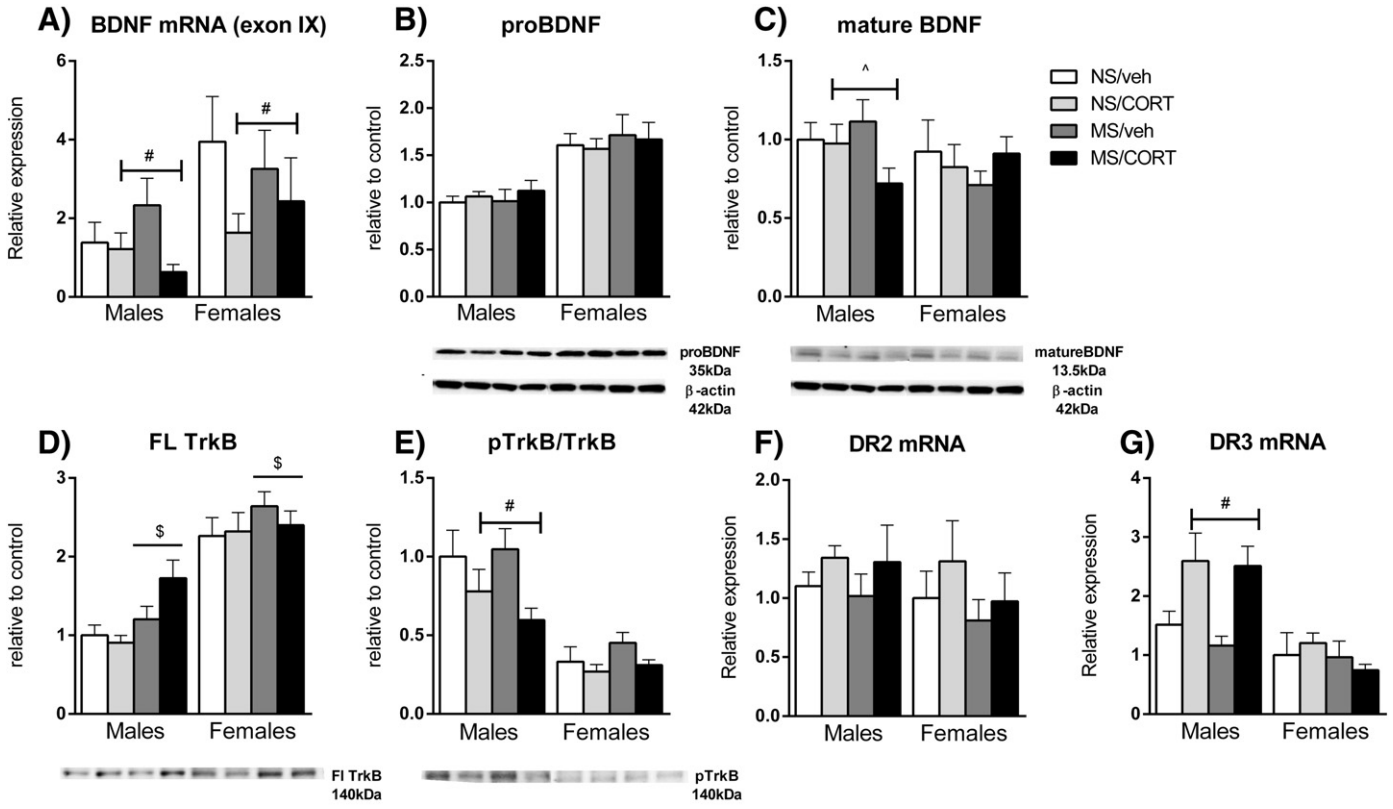

Fig. 5. BDNF-TrkB expression and signalling and dopamine receptor expression in the NAc. (A) BDNF mRNA levels, (B) proBDNF protein levels, (C) Mature BDNF protein levels, (D) Full-length TrkB protein levels, (E) Phosphorylated TrkB / full-length TrkB protein levels, (F) DR3 mRNA levels, (G) DR2 mRNA levels. ND/veh = non-deprived/vehicle-treated, ND/CORT = non-deprived/corticosterone-treated, MS/veh = maternally-deprived/vehicle-treated, and MS/CORT = maternally-deprived/corticosterone-treated. # over a horizontal line with vertical bars at either end = significant effects of CORT treatment, $ over a horizontal line = significant effects of MS, */#/$ = $P < 0.05$. ^ = $P < 0.1$, n = 5–6/group.

exposure stress had no immediate effect on MPFC BDNF protein expression in adult rats [50].

The observation, that the combined effect of two 'hits' on BDNF mRNA and protein in the mPFC was specific to males, may suggest a role for sex steroids hormones, such as testosterone or estrogen, in modulating this stress response. Indeed, the BDNF gene does contain a sequence with close homology to the estrogen response element (ERE) in its protein coding region, and BDNF and estrogen share converging downstream signalling pathways [51]. In addition to the male-specific 'two hit' effect on BDNF, we found a similar increase in DR3 expression and a significant, positive correlation between BDNF mRNA and DR3 mRNA expression. This is consistent with the literature; an acute exposure to cocaine produced a long-lasting increase in total BDNF mRNA and DR3 expression in the prefrontal cortex of rats [52] and a recent report demonstrated a similar positive correlation between BDNF protein and mRNA and DR3 mRNA levels in the prefrontal cortex of rats at 20, 35, 40 and 60 days of age [53]. Furthermore, Andersen et al. also showed that treatment with the DR3-preferring agonist, 7-OHDPAT, increased BDNF mRNA levels in the prefrontal cortex, suggesting that alterations in DR3 may be driving changes in BDNF expression [53]. Given the lack of change in TrkB expression and phosphorylation in the mPFC we would also propose, as above, that it is DR3 that may be driving changes in BDNF expression. Deletion of the DR3 receptor has been shown to increase levels of the tissue plasminogen activator (tPA), the enzyme responsible for cleavage of pro-BDNF to mature BDNF, in the prefrontal cortex of mice, and consequently leads to alterations in total BDNF mRNA and protein levels [54]. This may represent a mechanism by which alterations to DR3 lead to alterations in BDNF expression.

Table 3
Exon-specific BDNF transcript expression in the nucleus accumbens of male and female rats. Data represent mean percentage fold-change from the non-separated/vehicle-treated male control group ± SEM.

Fold-change from male controls								
Transcript	Males			Females				
	CORT	MS	MS+CORT	Control	CORT	MS	MS+CORT	Effect
BDNF exon I	1.26 ± 0.53	3.7 ± 1.2	0.96 ± 0.44	3.6 ± 2.3	1.2 ± 0.60	1.9 ± 0.47	4.6 ± 1.6	↑MS, $P = 0.04$
BDNF exon II	1.0 ± 0.28	1.4 ± 0.41	0.59 ± 0.04	2.8 ± 0.87	0.89 ± 0.20	1.3 ± 0.22	1.60 ± 0.5	0
BDNF exon III	1.1 ± 0.33	1.9 ± 0.56	0.61 ± 0.12	4.2 ± 1.33	1.1 ± 0.40	2.4 ± 0.56	2.6 ± 0.94	Sex, $P = 0.013$/CORT, $P = 0.045$
BDNF exon IV	0.99 ± 0.36	1.9 ± 0.73	0.46 ± 0.13	4.2 ± 1.46	1.4 ± 0.52	2.6 ± 0.68	3.1 ± 1.1	Sex, $P = 0.035$/CORT, $P = 0.043$
BDNF exon VI	1.2 ± 0.42	1.7 ± 0.61	0.43 ± 0.11	3.1 ± 1.00	1.2 ± 0.44	2.6 ± 0.69	2.2 ± 0.73	0
BDNF exon VII	1.2 ± 0.47	1.5 ± 0.65	0.25 ± 0.07	2.8 ± 0.90	0.82 ± 0.33	1.9 ± 0.51	2.0 ± 0.68	0
BDNF exon VIII	1.2 ± 0.51	1.2 ± 0.53	0.23 ± 0.06	2.4 ± 0.85	0.73 ± 0.26	1.5 ± 0.40	1.6 ± 0.56	0
BDNF exon IXa	0.90 ± 0.29	0.68 ± 0.32	0.31 ± 0.08	2.3 ± 0.78	0.84 ± 0.31	1.8 ± 0.48	1.6 ± 0.54	Sex, $P = 0.038$
BDNF exon IX	1.2 ± 0.40	2.3 ± 0.68	0.63 ± 0.19	3.9 ± 1.15	1.6 ± 0.48	3.2 ± 0.98	2.40 ± 1.15	Sex, $P = 0.049$/CORT, $P = 0.019$

MS = maternal separation, CORT = corticosterone treatment; ↑ indicates main effect as analysed by ANOVA.

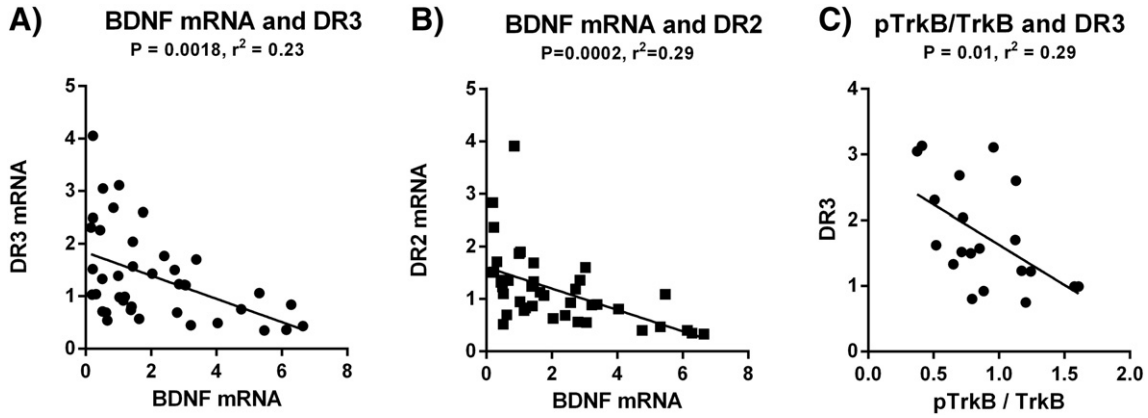

Fig. 6. Linear regression graphs showing correlation analysis of (A) BDNF mRNA (*x* axis) and DR3 mRNA (*y* axis) in the NAc, (B) BDNF mRNA (*x* axis) and DR2 mRNA (*y* axis) levels in the NAc and (C) TrkB phosphorylation/full-length TrkB (*x* axis) and DR3 (*y* axis) expression in the NAc.

While a trend for a correlation was noted between DR2 mRNA expression and BDNF mRNA expression, we did find a significant positive correlation between protein expression of both pro- and mature BDNF and DR2 in the mPFC. Previous reports have shown that blockade of dopamine receptors (predominantly DR2) via 3 days of treatment with haloperidol down-regulates BDNF protein expression in the prefrontal cortex, confirming a positive correlation between DR2 activation and BDNF expression [55].

Male-specific 'two hit' effects were also noted in the CPu, where although BDNF mRNA and protein levels were unchanged, TrkB expression was increased and TrkB phosphorylation was decreased. This may be a compensatory response to the increased BDNF protein expression in the mPFC. Indeed, we previously demonstrated an inverse relationship between mature BDNF protein levels and TrkB protein phosphorylation in the striatum of BDNF heterozygous mice whereby mature BDNF levels were reduced, but TrkB phosphorylation was increased [56]. Levels of BDNF mRNA have previously been reported to be low in the CPu; however, this region receives extensive projections from cortical pyramidal neurons that can anterogradely transport mature BDNF protein [57]. In addition, region-specific knockdown of BDNF in the prelimbic, but not orbitofrontal cortex, caused a reduction in striatal BDNF protein expression [58]. TrkB is abundantly expressed in the CPu [59], and we suggest that alterations to TrkB expression and phosphorylation in the CPu of male 'two hit' rats are due to increased anterograde transport of BDNF from the mPFC.

In the NAc region, CORT treatment reduced BDNF mRNA but increased DR3 expression, leading to a significant negative correlation between BDNF and DR3 expression. A negative correlation was also shown between TrkB phosphorylation and DR3 expression in males only. This finding was unexpected as earlier reports suggest that BDNF drives DR3 expression in the NAc [20] through activation of TrkB [60]. We suggest that perhaps in the face of chronic stress this mechanism is disrupted and potentially reversed. Previous studies have shown that CORT treatment in rats enhances dopamine release in the NAc [61]. In addition, high stress-reactive mice show increased expression of DR2 in the nucleus accumbens [62]. Our results suggest that the mechanism by which chronic CORT treatment up-regulates DR2 and DR3 expression may be via long-term alterations to BDNF-TrkB signalling.

We have previously investigated behavioural changes in a comparable 'two hit' rat model and observed an altered dopaminergic regulation of prepulse inhibition (PPI) in male rats. Therefore it is tempting to speculate that the altered expression profile of BDNF, DR2 and DR3 in male two-hit rats shown in the current study may, at least in part, explain the previously found altered PPI response to dopamine receptor stimulation by apomorphine and amphetamine [34]. Our previous studies reported no significant effect of either 'hits' on DR1 or DR2 receptor expression in the CPu or NAc using receptor autoradiography [34]. Our current study supports and extends this finding in that we found no significant changes in DR2 receptor expression in the CPu or NAc. However, we did find a significant effect of CORT treatment on DR3 expression in the NAc, as well as a significant two-hit effect on both DR2 and DR3 expression in the mPFC.

It is important to note that the majority of BDNF in the NAc is derived from the ventral tegmental area (VTA). A recent study by Walsh et al. [63] demonstrated that BDNF expression in the NAc was up-regulated following phasic optogenetic stimulation of the VTA-NAc pathway, in socially stressed, but not stress-naïve mice. This stress-dependent up-regulation of BDNF was mediated via corticotrophin releasing factor (CRF), which, if blocked using a CRF receptor antagonist, prevented BDNF up-regulation [63]. While Walsh et al. found that social stress alone had no effect on BDNF expression, our results in the NAc show that chronic CORT treatment during adolescence caused a long-lasting reduction in BDNF expression in the NAc. Given the role of CRF in mediating stress-induced alterations in BDNF expression in the NAc, future studies should examine the effects of CRF receptor antagonist treatment in our two-hit rat model. Behaviourally, Walsh et al. showed reduced social interaction in socially-stressed, but not stress-naive VTA-NAc stimulated animals [63]. Therefore social behaviour would also be of interest in future two-hit rat model studies.

In summary, we report that the combined effect of two stressors during critical periods of brain development causes a significant, long-lasting up-regulation of BDNF mRNA and protein levels in the mPFC of male, but not female rats, and this correlated significantly with an up-regulation of DR3 mRNA expression. In addition, we show here that chronic CORT treatment reduces BDNF mRNA expression in the NAc, but up-regulates DR3 expression in the NAc of male rats. This is in contrast to normal development, whereby BDNF is known to promote DR3 expression. These data provide further insight into the impact of stress at critical time periods of brain development.

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References

[1] M.J. Green, S.L. Matheson, A. Shepherd, C.S. Weickert, V.J. Carr, Brain-derived neurotrophic factor levels in schizophrenia: a systematic review with meta-analysis, Mol. Psychiatry 16 (2011) 960–972.

[2] M. Gratacos, J.R. Gonzalez, J.M. Mercader, R. de Cid, M. Urretavizcaya, X. Estivill, Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia, Biol. Psychiatry 61 (2007) 911–922.

[3] C.S. Weickert, D.L. Ligons, T. Romanczyk, G. Ungaro, T.M. Hyde, M.M. Herman, D.R. Weinberger, J.E. Kleinman, Reductions in neurotrophin receptor mRNAs in the prefrontal cortex of patients with schizophrenia, Mol. Psychiatry 10 (2005) 637–650.

[4] C.S. Weickert, T.M. Hyde, B.K. Lipska, M.M. Herman, D.R. Weinberger, J.E. Kleinman, Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia, Mol. Psychiatry 8 (2003) 592–610.

[5] J. Wong, T.M. Hyde, H.L. Cassano, A. Deep-Soboslay, J.E. Kleinman, C.S. Weickert, Promoter specific alterations of brain-derived neurotrophic factor mRNA in schizophrenia, Neuroscience 169 (2010) 1071–1084.

[6] T. Aid, A. Kazantseva, M. Piirsoo, K. Palm, T. Timmusk, Mouse and rat BDNF gene structure and expression revisited, J. Neurosci. Res. 85 (2007) 525–535.

[7] G. Baj, D. Del Turco, J. Schlaudraff, L. Torelli, T. Deller, E. Tongiorgi, Regulation of the spatial code for BDNF mRNA isoforms in the rat hippocampus following pilocarpine-treatment: a systematic analysis using laser microdissection and quantitative real-time PCR, Hippocampus 23 (2013) 413–423.

[8] To Timmusk, M. Metsis, Regulation of BDNF promoters in the rat hippocampus, Neurochem. Int. 25 (1994) 11–15.

[9] F. Sohrabji, R.C. Miranda, C.D. Toran-Allerand, Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 11110–11114.

[10] A. Nair, K.C. Vadodaria, S.B. Banerjee, M. Benekareddy, B.G. Dias, R.S. Duman, V.A. Vaidya, Stressor-specific regulation of distinct brain-derived neurotrophic factor transcripts and cyclic AMP response element-binding protein expression in the postnatal and adult rat hippocampus, Neuropsychopharmacology 32 (2007) 1504–1519.

[11] V.Y. Hayes, M.D. Towner, P.J. Isackson, Organization, sequence and functional analysis of a mouse BDNF promoter, Brain Res. Mol. Brain Res. 45 (1997) 189–198.

[12] M.S. Zajac, T.Y. Pang, N. Wong, B. Weinrich, L.S. Leang, J.M. Craig, R. Saffery, A.J. Hannan, Wheel running and environmental enrichment differentially modify exon-specific BDNF expression in the hippocampus of wild-type and pre-motor symptomatic male and female Huntington's disease mice, Hippocampus 20 (2010) 621–636.

[13] J.C. Lauterborn, F.R. Poulsen, C.T. Stinis, P.J. Isackson, C.M. Gall, Transcript-specific effects of adrenalectomy on seizure-induced BDNF expression in rat hippocampus, Brain Res. Mol. Brain Res. 30 (55) (1998) 81–91.

[14] Y. Dwivedi, H.S. Rizavi, G.N. Pandey, Antidepressants reverse corticosterone-mediated decrease in brain-derived neurotrophic factor expression: differential regulation of specific exons by antidepressants and corticosterone, Neuroscience 139 (2006) 1017–1029.

[15] M.E. Greenberg, B. Xu, B. Lu, B.L. Hempstead, New insights in the biology of BDNF synthesis and release: implications in CNS function, J. Neurosci. 29 (2009) 12764–12767.

[16] E.J. Huang, L.F. Reichardt, Trk receptors: roles in neuronal signal transduction, Ann. Rev. Biochem. 72 (2003) 609–642.

[17] E.J. Hess, H.S. Bracha, J.E. Kleinman, I. Creese, Dopamine receptor subtype imbalance in schizophrenia, Life Sci. 40 (1987) 1487–1497.

[18] L. Depatie, S. Lal, Apomorphine and the dopamine hypothesis of schizophrenia: a dilemma? J. Psychiatry Neurosci. 26 (2001) 203–220.

[19] N. Ginovart, S. Kapur, Role of dopamine D_2 receptors for antipsychotic activity, Handb. Exp. Pharmacol. (2012) 27–52.

[20] O. Guillin, J. Diaz, P. Carroll, N. Griffon, J.C. Schwartz, P. Sokoloff, BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization, Nature 411 (2001) 86–89.

[21] A. Hasbi, T. Fan, M. Alijaniaram, T. Nguyen, M.L. Perreault, B.F. O'Dowd, S.R. George, Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 21377–21382.

[22] L. De Carolis, M.A. Stasi, O. Serlupi-Crescenzi, F. Borsini, P. Nencini, The effects of clozapine on quinpirole-induced non-regulatory drinking and prepulse inhibition disruption in rats, Psychopharmacology 212 (2010) 105–115.

[23] F. Angelucci, A.A. Mathe, L. Aloe, Brain-derived neurotrophic factor and tyrosine kinase receptor TrkB in rat brain are significantly altered after haloperidol and risperidone administration, J. Neurosci. Res. 60 (2000) 783–794.

[24] T.M. Maynard, L. Sikich, J.A. Lieberman, A.S. LaMantia, Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia, Schizophr. Bull. 27 (2001) 457–476.

[25] B.A. Ellenbroek, P.T. van den Kroonenberg, A.R. Cools, The effects of an early stressful life event on sensorimotor gating in adult rats, Schizophr. Res. 30 (1998) 251–260.

[26] J. Chen, Z. Wang, M. Li, Multiple 'hits' during postnatal and early adulthood periods disrupt the normal development of sensorimotor gating ability in rats, J. Psychopharmacol. 25 (2011) 379–392.

[27] D. Suri, V. Veenit, A. Sarkar, D. Thiagarajan, A. Kumar, E.J. Nestler, S. Galande, V.A. Vaidya, Early stress evokes age-dependent biphasic changes in hippocampal neurogenesis, BDNF expression, and cognition, Biol. Psychiatry 73 (2013) 658–666.

[28] K.Y. Lee, T. Miki, T. Yokoyama, et al., Neonatal repetitive maternal separation causes long-lasting alterations in various neurotrophic factor expression in the cerebral cortex of rats, Life Sci. 90 (2012) 578–584.

[29] S.L. Gourley, A.M. Swanson, A.M. Jacobs, J.L. Howell, M. Mo, R.J. Dileone, A.J. Koleske, J.R. Taylor, Action control is mediated by prefrontal BDNF and glucocorticoid receptor binding, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 20714–20719.

[30] S.L. Gourley, A.T. Kedves, P. Olausson, J.R. Taylor, A history of corticosterone exposure regulates fear extinction and cortical NR2B, GluR2/3, and BDNF, Neuropsychopharmacology 34 (2009) 707–716.

[31] K.A. Butts, J. Weinberg, A.H. Young, A.G. Phillips, Glucocorticoid receptors in the prefrontal cortex regulate stress-evoked dopamine efflux and aspects of executive function, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 18459–18464.

[32] C.H. Lammers, U.M. D'Souza, Z.H. Qin, S.H. Lee, S. Yajima, M.M. Mouradian, Regulation of striatal dopamine receptors by corticosterone: an in vivo and in vitro study, Brain Res. Mol. Brain Res. 69 (1999) 281–285.

[33] K.H. Choy, Y. de Visser, N.R. Nichols, M. van den Buuse, Combined neonatal stress and young-adult glucocorticoid stimulation in rats reduce BDNF expression in hippocampus: effects on learning and memory, Hippocampus 18 (2008) 655–667.

[34] K.H. Choy, Y.P. de Visser, M. van den Buuse, The effect of 'two hit' neonatal and young-adult stress on dopaminergic modulation of prepulse inhibition and dopamine receptor density, Br. J. Pharmacol. 156 (2009) 388–396.

[35] R.A. Hill, M. Klug, S. Kiss Von Soly, M.D. Binder, A.J. Hannan, M. van den Buuse, Sex-specific disruptions in spatial memory and anhedonia in a "two hit" rat model correspond with alterations in hippocampal brain-derived neurotrophic factor expression and signaling, Hippocampus (2013), http://dx.doi.org/10.1002/hipo.22302 [Epub ahead of print].

[36] M.T. Ray, C. Shannon Weickert, M.J. Webster, Decreased BDNF and TrkB mRNA expression in multiple cortical areas of patients with schizophrenia and mood disorders, Transl. Psychiatry 4 (2014) e389.

[37] S.S. Kaalund, E.N. Newburn, T. Ye, et al., Contrasting changes in DRD1 and DRD2 splice variant expression in schizophrenia and affective disorders, and associations with SNPs in postmortem brain, Mol. Psychiatry (2013), http://dx.doi.org/10.1038/mp.2013.165 [Epub ahead of print].

[38] J. Brunelin, S. Fecteau, M.F. Suaud-Chagny, Abnormal striatal dopamine transmission in schizophrenia, Curr. Med. Chem. 20 (2013) 397–404.

[39] S.M. Perez, D.J. Lodge, Aberrant dopamine D2-like receptor function in a rodent model of schizophrenia, J. Pharmacol. Exp. Ther. 343 (2012) 288–295.

[40] M.L. Santoro, V.K. Ota, R.S. Stilhano, et al., Effect of antipsychotic drugs on gene expression in the prefrontal cortex and nucleus accumbens in the spontaneously hypertensive rat (SHR), Schizophr. Res. 157 (2014) 163–168.

[41] G. Paxinos, C. Watson, The Rat Brain in stereotaxic coordinates, 5th ed. Elsevier Academic Press, 2005.

[42] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method, Methods 25 (2001) 402–408.

[43] J. Wong, M.J. Webster, H. Cassano, C.S. Weickert, Changes in alternative brain-derived neurotrophic factor transcript expression in the developing human prefrontal cortex, Eur. J. Neurosci. 29 (2009) 1311–1322.

[44] C.L. Burton, D. Chatterjee, M. Chatterjee-Chakraborty, V. Lovic, S.L. Grella, M. Steiner, A.S. Fleming, Prenatal restraint stress and motherless rearing disrupts expression of plasticity markers and stress-induced corticosterone release in adult female Sprague-Dawley rats, Brain Res. 1158 (2007) 28–38.

[45] Y. Zhao, B. Xing, Y.H. Dang, C.L. Qu, F. Zhu, C.X. Yan, Microinjection of valproic acid into the ventrolateral orbital cortex enhances stress-related memory formation, PLoS One 8 (2013) e52698.

[46] Y. Lee, R.S. Duman, G.J. Marek, The mGlu2/3 receptor agonist LY354740 suppresses immobilization stress-induced increase in rat prefrontal cortical BDNF mRNA expression, Neurosci. Lett. 398 (2006) 328–332.

[47] R.M. de Pablos, R.F. Villaran, S. Arguelles, A.J. Herrera, J.L. Venero, A. Ayala, J. Cano, A. Machado, Stress increases vulnerability to inflammation in the rat prefrontal cortex, J. Neurosci. 26 (2006) 5709–5719.

[48] S.T. Bland, J.P. Tamlyn, R.M. Barrientos, B.N. Greenwood, L.R. Watkins, S. Campeau, H. E. Day, S.F. Maier, Expression of fibroblast growth factor-2 and brain-derived neurotrophic factor mRNA in the medial prefrontal cortex and hippocampus after uncontrollable or controllable stress, Neuroscience 144 (2007) 1219–1228.

[49] B. Ray, D.L. Gaskins, T.J. Sajdyk, J.P. Spence, S.D. Fitz, A. Shekhar, D.K. Lahiri, Restraint stress and repeated corticotrophin-releasing factor receptor activation in the amygdala both increase amyloid-beta precursor protein and amyloid-beta peptide but have divergent effects on brain-derived neurotrophic factor and pre-synaptic proteins in the prefrontal cortex of rats, Neuroscience 184 (2011) 139–150.

[50] P.R. Zoladz, C.R. Park, J.D. Halonen, et al., Differential expression of molecular markers of synaptic plasticity in the hippocampus, prefrontal cortex, and amygdala in response to spatial learning, predator exposure, and stress-induced amnesia, Hippocampus 22 (2012) 577–589.

[51] R.A. Hill, Interaction of sex steroid hormones and brain-derived neurotrophic factor-tyrosine kinase B signalling: relevance to schizophrenia and depression, J. Neuroendocrinol. 24 (2012) 1553–1561.

[52] B. Le Foll, J. Diaz, P. Sokoloff, A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning, Neuroreport 16 (2005) 175–178.

[53] S.L. Andersen, K.C. Sonntag, Juvenile methylphenidate reduces prefrontal cortex plasticity via D3 receptor and BDNF in adulthood, Front. Synaptic Neurosci. 6 (2014) 1.

[54] A. Castorina, A.G. D'Amico, S. Scuderi, G.M. Leggio, F. Drago, V. D'Agata, Dopamine D3 receptor deletion increases tissue plasminogen activator (tPA) activity in prefrontal cortex and hippocampus, Neuroscience 250 (2013) 546–556.
[55] N.M. Dawson, E.H. Hamid, M.F. Egan, G.E. Meredith, Changes in the pattern of brain-derived neurotrophic factor immunoreactivity in the rat brain after acute and subchronic haloperidol treatment, Synapse 39 (2001) 70–81.
[56] R.A. Hill, M. van den Buuse, Sex-dependent and region-specific changes in TrkB signaling in BDNF heterozygous mice, Brain Res. 1384 (2011) 51–60.
[57] C.A. Altar, N. Cai, T. Bliven, M. Juhasz, J.M. Conner, A.L. Acheson, R.M. Lindsay, S.J. Wiegand, Anterograde transport of brain-derived neurotrophic factor and its role in the brain, Nature 389 (1997) 856–860.
[58] S.L. Gourley, J.L. Howell, M. Rios, R.J. DiLeone, J.R. Taylor, Prelimbic cortex bdnf knock-down reduces instrumental responding in extinction, Learn. Mem. 16 (2009) 756–760.
[59] A.Y. Freeman, J.J. Soghomonian, R.C. Pierce, Tyrosine kinase B and C receptors in the neostriatum and nucleus accumbens are co-localized in enkephalin-positive and enkephalin-negative neuronal profiles and their expression is influenced by cocaine, Neuroscience 117 (2003) 147–156.
[60] J. Liang, X. Zheng, J. Chen, Y. Li, X. Xing, Y. Bai, Roles of BDNF, dopamine D_3 receptors, and their interactions in the expression of morphine-induced context-specific locomotor sensitization, Eur. Neuropsychopharmacol. 21 (2011) 825–834.
[61] E.N. Graf, R.A. Wheeler, D.A. Baker, et al., Corticosterone acts in the nucleus accumbens to enhance dopamine signaling and potentiate reinstatement of cocaine seeking, J. Neurosci. 17 (33) (2013) 11800–11810.
[62] A. Knapman, J.M. Heinzmann, F. Holsboer, R. Landgraf, C. Touma, Modeling psychotic and cognitive symptoms of affective disorders: disrupted latent inhibition and treversal learning deficits in highly stress reactive mice, Neurobiol. Learn. Mem. 94 (2010) 145–152.
[63] J.J. Walsh, A.K. Friedman, H. Sun, et al., Stress and CRF gate neural activation of BDNF in the mesolimbic reward pathway, Nat. Neurosci. 17 (2014) 27–29.