Region-specific effects on BDNF expression after contingent or non-contingent cocaine i.v. self-administration in rats

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Abstract

Brain-derived neurotrophic factor (BDNF) dynamic changes were investigated in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) during use and the early phases of cocaine abstinence after 14 sessions (2 h self-administration/d; 0.25 mg/0.1 ml 6 s infusion) by employing a ‘yoked control-operant paradigm’. The effect on BDNF was region-specific and dependent on the withdrawal time. In the NAc, BDNF protein levels increased immediately after the last self-administration session, with a larger increase in passively cocaine-exposed rats. In the mPFC, BDNF expression was elevated 24 h after the last self-administration session, independently of how the drug was encountered. No changes were found in NAc and mPFC 7 d after the last self-administration session. Analysis of transcript levels in the mPFC indicated that action on exon I might contribute to BDNF’s cortical induction. These findings indicate a finely tuned modulation of BDNF expression during use and early phases of cocaine abstinence.

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Introduction

An essential step to develop therapeutic approaches for cocaine addiction is to understand the cellular mechanisms underlying the craving and relapse after withdrawal. Growing evidence suggests that cocaine-induced alterations of brain-derived neurotrophic factor (BDNF) mRNA and protein levels in mesocorticolimbic nuclei are vital in cocaine craving and relapse (Ghitza et al. 2010; McGinty et al. 2010). In fact, cue-induced cocaine-seeking progressively increased during protracted abstinence, paralleled by up-regulation of BDNF protein levels in the ventral tegmental area (VTA), nucleus accumbens (NAc) and amygdala (Grimm et al. 2003). Moreover, repeated cocaine self-administration increased BDNF protein levels in the medial prefrontal cortex (mPFC) and VTA after 7 d of abstinence (Sadri-Vakili et al. 2010; Schmidt et al. 2012). In addition, a single infusion of BDNF into the mPFC or VTA suppressed the reinstatement of drug-seeking by normalizing cocaine-induced alterations in NAc glutamate transmission (Berglind et al. 2007, 2009; McGinty et al. 2010), whereas single or repeated infusions into the NAc and VTA promoted or facilitated cocaine-seeking behaviour (Graham et al. 2007; Lu et al. 2004).

These findings suggest that self-administered cocaine alters BDNF expression primarily as a result of prolonged drug withdrawal (Grimm et al. 2003; Sadri-Vakili et al. 2010). However, Graham et al. (2007) showed an immediate, but transient, increase in BDNF levels in the NAc, suggesting that modulation of BDNF expression after drug withdrawal needs further investigation. Thus, the aim of our study was to investigate: (1) whether dynamic changes of BDNF levels also occurred in early abstinence; (2) whether such changes rely on the contingency of cocaine self-administration. This is important in light of evidence that acute and chronic cocaine use, even when passively administered, alters BDNF expression in the rat brain (Filip et al. 2006; Fumagalli et al. 2007; Le Foll et al. 2005). To this aim, by employing the ‘yoked control-operant paradigm’, in which each rat actively taking cocaine is paired with two yoked controls receiving passive infusions of cocaine or saline (Fumagalli et al. 2009b; Jacobs et al. 2003), we evaluated the dynamic regulation...
of BDNF expression during use and the early phases of cocaine abstinence after repeated sessions of cocaine self-administration, killing the animals immediately, 24 h or 7 d after the last session.

Materials and method

Animals

Animal procedures were conducted in conformity to institutional guidelines and national policies (EEC Directive 86/609; Guide for the Care and Use of Laboratory Animals, USA). Naive male Sprague–Dawley CD®IGS rats (Charles River, Italy), weighing 250–280 g, were housed individually in a specific pathogen-free vivarium at 21 ± 1 °C and 60% humidity under a 12 h light/dark cycle (lights on 07:30 hours) with food and water freely available. They were allowed to adapt to these conditions for 2 wk and handled daily during this period. After this, to prevent excessive weight gain they were fed 20 g/animal.d of rat chow in the evening. Rats on this diet gain weight at 1–3 g/d.

Cocaine self-administration

Self-administration started 7 d after the jugular catheters implantation (Cervo et al. 2007; Di Clemente et al. 2012). Rats were randomly assigned to three groups of triads, cocaine self-administration (SA), yoked cocaine (YC) and yoked saline (YS), to be killed immediately, 24 h or 7 d after the last self-administration session. The 2-h SA sessions/d were conducted 7 d/wk for 14 d during the dark phase of the light/dark cycle. The SA procedure has been described in detail previously (Fumagalli et al. 2009b). Briefly, three littermates were trained at the same time. They were individually placed in operant chambers (MED Associates Ltd, USA) controlled by a computer with MED-software. Sessions started with the introduction of two levers and illumination of the house light. The SA rat of each triad was allowed to self-administer i.v. cocaine (0.25 mg/0.1 ml 6 s infusion; MacFarlan Smith, UK) on a fixed-ratio-1 schedule. Infusions were made by an infusion pump, through a swivel device mounted above the chamber and connected to the external guide cannulae of the catheter. Each infusion was followed by the house light turn-off, retraction of levers and illumination of the stimulus light above the active lever, all of which reverted after 20 s. Further depression of the active lever triggered the same sequence of events. Pressure on the other, inactive lever had no programmed consequences. In half the chambers, the right-hand lever was active, in the others the left-hand one. Cocaine hydrochloride solution was freshly re-mixed every 4 d, filtered through a 20-μm syringe filter and stored at 4 °C.

The other two littermates received a response-independent infusion either of the same dose of cocaine (YC) or saline (YS) accompanied by the same sequence of events as above, whenever the SA rat did. At the scheduled times after the last self-administration, rats were killed by decapitation.

Their brains were quickly removed and the mPFC (including cingulate, infralimbic, prelimbic cortex sub-regions) was quickly dissected on ice from a 2 mm coronal section extending from approximately bregma +5.16 to +3.24 (Paxinos & Watson, 2005). The NAc (including NAc shell and NAc core subregions) was dissected from a 2 mm section immediately caudal to the mPFC section. Tissues were immediately frozen on dry ice and stored at −80 °C until assay.

Preparation of protein extracts and Western blot analysis

The preparation of the homogenate and crude synaptic-somal fractions from mPFC and NAc as well as the Western blot procedure was as previously described (Fumagalli et al. 2009a). Total protein content (BDNF precursor and mature forms) was measured using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Italy). Total protein concentrations were adjusted to the same amount for all samples (10 μg/lane). BDNF precursor (proBDNF) and mature BDNF (mBDNF) were detected from the band density at 32 and 14 kDa, respectively, after probing with a polyclonal antibody (1:500, overnight, 4 °C; Santa Cruz Biotechnology, USA). Results were standardized using β-actin as a housekeeping protein, detected by examining the band density at 43 kDa after probing with a monoclonal antibody (1:10 000; Sigma, Italy). The protein immunocomplexes were visualized by chemiluminescence utilizing the ECL Western Blotting kit (Amersham Life Science, Italy).

Analysis of gene expression

RNA extraction and real-time polymerase chain reaction were performed as previously described (Fumagalli et al. 2012). Samples were run in 384-well formats in triplicate as multiplexed reactions with a normalizing internal control (36B4). The TaqMan Gene Expression Assays (Applied Biosystem, Italy) used for total BDNF and BDNF transcripts I, IV and VI were as previously described (Fumagalli et al. 2012).

Statistical analysis

The numbers of active and inactive lever presses were analysed by mixed-factorial analysis of variance (ANOVA) with modality of self-administration (SA, YC, YS) and sessions as between- and within-subject factors, respectively. The amount of cocaine (mg/kg i.v.) self-administered during the 14 sessions was analysed by mixed-factorial ANOVA using, respectively, the time of death and sessions as between- and within-subjects factors. Molecular data were analysed by one-way ANOVA with modality of cocaine exposure as main factor. When
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either the mPFC or NAc (data not shown). mBDNF protein levels were high in the whole homogenate of NAc in SA and YC rats immediately after the last self-administration session ($F_{2,15} = 18.2, p < 0.001, p < 0.05$ and $p < 0.001$, respectively, vs. YS, N–K test; Fig. 2a). The increase was significantly larger in YC rats ($p < 0.01$ vs. SA, N–K test). Analysis of the crude synaptosomal fraction indicated significant up-regulation of BDNF protein levels in the NAc of YC animals ($F_{2,11} = 7.6, p < 0.01; p < 0.05$ vs. YS and $p < 0.01$ vs. SA, N–K test; Fig. 2a).

mBDNF protein levels were not altered in the mPFC of SA and YC rats killed immediately after the last self-administration session (Fig. 2b). There were no changes of mBDNF protein in the NAc of SA and YC rats killed after 24-h withdrawal (Fig. 2c). Figure 2d shows that mBDNF protein levels were raised in the crude synaptosomal fraction of mPFC of the SA and YC rats after 24-h withdrawal ($F_{2,15} = 9.6, p < 0.01; p < 0.01$ vs. YS, N–K test); in the homogenate, SA and YC groups showed a non-significant tendency toward an increase. After 7 d abstinence, BDNF protein levels were similar in NAc or mPFC of SA, YC and YS rats (data not shown).

To investigate whether changes in BDNF protein levels were due to an effect on BDNF mRNA levels, we measured total and BDNF transcripts. The BDNF gene is characterized by several 5′ non-coding exons, each with a separate promoter region driving the transcription of a common 3′ exon and encoding for the same protein (Aid et al. 2007). Although their roles are obscure, it would be informative to investigate their modulation under these experimental conditions. Since BDNF mRNA is absent in the NAc (Altar et al. 1997; Conner et al. 1997), we focused on mPFC after 24-h withdrawal, when protein levels were raised. There were no significant changes in total BDNF mRNA levels (data not shown). Figure 2e shows that BDNF exon I mRNA levels were high in the mPFC of SA and YC rats ($F_{2,11} = 5.4, p < 0.05; p < 0.05$ vs. YS, N–K test). Figure 2f shows the low BDNF exon IV mRNA levels in the mPFC of SA and YC rats ($F_{2,11} = 9.4, p < 0.01; p < 0.01$ vs. YS, N–K test). BDNF exon VI mRNA levels were unchanged in the mPFC of SA and YC rats (data not shown).

Discussion

Our findings indicate that BDNF protein expression in the NAc and mPFC during the early phases of abstinence is a dynamic phenomenon whose brain location is regulated respectively by the presence of and abstinence from cocaine. In the mPFC these effects appear to be qualitatively independent from the modality of cocaine exposure, suggesting that BDNF changes in this region do not depend on how the drug is encountered, whereas in the NAc the different modalities of exposure seem to affect BDNF protein levels differently. The timing of BDNF activation was different in the cortico–accumbal pathway. BDNF protein levels

![Graph](https://academic.oup.com/ijnp/article-abstract/16/4/913/791919/1643137919)
increased in the NAc immediately after the last self-administration session, whereas in the mPFC the increase was observed 24 h later. In the NAc, both contingent and non-contingent cocaine raised BDNF protein levels, reflecting a generalized pharmacological response to cocaine in line with the results from Graham et al. (2007). However, BDNF protein levels were significantly higher in the NAc of YC rats, an effect that appeared to be localized in the crude synaptosomal fraction (unlike in SA animals, where it seems to occur in the cytosol; data
not shown), suggesting that cocaine boosts the pool of BDNF ready to be released. Since no changes in proBDNF levels were observed in either group, the larger increase in YC rats’ mBDNF might be indicative of increased processing of the neurotrophin.

This increase, as seen immediately but not 24 h after cocaine self-administration, in NAc-shell but not NAccore by Graham et al. (2007) suggests that dynamic regulation of BDNF synthesis in the NAc, probably in the NAc-shell subregion during ongoing cocaine use/exposure, may contribute to the development and maintenance of cocaine addiction.

At present, the larger increase of BDNF levels in YC rats is hard to explain. Although elevated BDNF are associated with enhancement of dopamine (DA) neurotransmission (McGinty et al. 2010), the larger increase in the YC rats should not be related to DA different efflux since higher DA extracellular levels were observed in the NAc of SA animals (Hemby et al. 1997; Lecca et al. 2007). It can be speculated that the stressful component associated with the non-contingent cocaine (Twinning et al. 2009) may contribute to the greater elevation of BDNF protein levels in YC rats, as repeated stress raises BDNF expression in this brain region (Berton & Nestler, 2006). However, considering the different effects on rats’ neurophysiology and behaviour induced by contingent and non-contingent cocaine, future research should address this important issue.

The picture was different in the mPFC, where BDNF protein levels were raised in the crude synaptosomal fraction of SA and YC rats after 24 h withdrawal. The analysis of BDNF expression at this time-point shows only a single evidence illustrating a decrease in BDNF mRNA levels in the mPFC (McGinty et al. 2010). However, evidence exists that exogenous infusion of the neurotrophin in the mPFC suppresses cocaine-seeking after 22 h abstinence, raising the possibility that the increase of BDNF protein in the mPFC might be an early adaptive strategy to oppose cocaine seeking and could be interpreted as a compensatory reaction, perhaps to normalize drug-induced alterations in glutamate transmission in the NAc (Berglind et al. 2009; McGinty et al. 2010; Sadri-Vakili et al. 2010).

The analysis of BDNF mRNA transcripts in mPFC showed that BDNF exon I levels were enhanced while BDNF exon IV levels were reduced after 24 h withdrawal. The high BDNF exon I mRNA levels might help to explain the non-significant increase of BDNF protein in the whole homogenate in both SA and YC rats, whereas the larger up-regulation in the crude synaptosomal fraction might reflect post-translational mechanisms that might occur in this membrane-enriched fraction. Overall, the reduction of BDNF exon IV mRNA, known to be regulated by neuronal activity (Pruunsild et al. 2011), together with the increased mBDNF expression in both mPFC and NAc may suggest a dysregulation of the BDNF system in the cortico-accumbal pathway.

We found no change in BDNF protein levels after 7 d withdrawal, in contrast with Sadri-Vakili et al. (2010) who found high BDNF protein levels at this time. This difference is hard to explain given the similar experimental conditions; however, there were some differences between the self-administration procedures. Sadri-Vakili’s rats received food ad libitum and self-administered cocaine during the light phase, whereas our rats were under a restricted food regimen and self-administered cocaine during the dark phase. Further, Sadri-Vakili’s rats started the 14 d self-administration when the animals achieved stable FR1 responding, whereas we started from the first self-administration session, implying a higher total intake of cocaine. Moreover, the dissection of the mPFC in two studies may have included different mPFC sub-regions. Finally, Sadri-Vakili’s rats may be more sensitive to cocaine, thus showing a longer increase in BDNF expression.

In conclusion, we found that cocaine self-administration affected BDNF expression in a way that depended on the presence of the psychostimulant in the NAc and on short abstinence in the mPFC. The contingency of cocaine self-administration also had different effects on BDNF protein levels in NAc and mPFC, suggesting that while in the mPFC the levels depend on the pharmacological properties of the drug, in the NAc other factors may come into play to drive BDNF changes.

Statement of Interest
None.

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References


