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ARTICLE

Prolonged abstinence from developmental cocaine exposure dysregulates BDNF and its signaling network in the medial prefrontal cortex of adult rats

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Abstract

Although evidence exists that chronic cocaine exposure during adulthood is associated with changes in BDNF expression, whether and how cocaine exposure during adolescence modulates BDNF is still unknown. To address this issue, we exposed rats to repeated cocaine injections from post-natal day (PD) 28 to PD 42, a period that roughly approximates adolescence in humans, and we carried out a detailed analysis of the BDNF system in the medial prefrontal cortex (mPFC) of rats sacrificed 3 d (PD 45) and 48 d (PD 90) after the last cocaine treatment. We found that developmental exposure to cocaine altered transcriptional and translational mechanisms governing neurotrophin expression. Total BDNF mRNA levels, in fact, were enhanced in the mPFC of PD 90 rats exposed to cocaine in adolescence, an effect sustained by changes in BDNF exon IV through the transcription factors CaRF and NF-kB. While a profound reduction of specific BDNF-related miRNAs (let7d, miR124 and miR132) may contribute to explaining the increased proBDNF levels, the up-regulation of the extracellular proteases tPA is indicative of increased processing leading to higher levels of released mBDNF. These changes were associated with increased activation of the trkB-Akt pathway resulting in enhanced pmTOR and pS6 kinase, which ultimately produced an up-regulation of Arc and a consequent reduction of GluA1 expression in the mPFC of PD 90 cocaine-treated rats. These findings demonstrate that developmental exposure to cocaine dynamically dysregulates BDNF and its signaling network in the mPFC of adult rats, providing novel mechanisms that may contribute to cocaine-induced changes in synaptic plasticity.

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Key words: Adolescence, cocaine, neurotrophins, prefrontal cortex.

Introduction

It is well established that adolescence is a vulnerable period of development characterized by a unique sensitivity to drug abuse (Kelley et al., 2004). In fact, during this period, the ongoing maturational changes (Spear, 2000; Casey et al., 2008; Ernst et al., 2009) make specific brain structures more sensitive to the effects of abused drugs (Andersen, 2003). However, the mechanisms underlying such higher sensitivity are still obscure.

Cocaine is a highly addictive psychostimulant, the abuse of which causes a great social and economic burden worldwide. Identifying the molecular mechanisms that might contribute to the craving and relapse after cocaine withdrawal represents, so far, an unmet need. Recent research has pointed to the neurotrophin BDNF as an important player in the action of cocaine. In fact, acute or repeated cocaine exposure increases BDNF expression in different brain regions (Filip et al., 2006; Fumagalli et al., 2007, 2009a, 2013; Sadri-Vakili et al., 2010), suggesting that the modulation of the neurotrophin expression may participate in the adaptations set in motion by exposure to the psychostimulant. The expression of BDNF is also increased as a function of abstinence; in fact, Grimm et al. (2003) have shown that after long-term withdrawal neurotrophin protein levels progressively increased in the nucleus accumbens (NAc), ventral tegmental area (VTA) and amygdala (Grimm et al., 2003), suggesting that BDNF plays an important role in cocaine craving.

In particular, it has been recently reported that BDNF has a protective role toward cocaine-mediated behaviours

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in the rat medial prefrontal cortex (mPFC). In fact, infusion of the neurotrophin in the mPFC suppresses cocaine seeking (Berglind et al., 2007), whereas BDNF infusion in the NAc or VTA increases cocaine seeking (Horger et al., 1999; Lu et al., 2004; Graham et al., 2007), suggesting that BDNF might be a pro- or anti-addictive factor, depending on the brain region of the infusion. Interestingly, mPFC and NAc are strictly connected in the regulation of BDNF, since Berglind et al. (2009) have shown that elevating BDNF levels in the mPFC normalizes cocaine-induced alterations in extracellular glutamate within the nucleus accumbens, providing a potential molecular mechanism for the BDNF-induced protection against cocaine seeking (Berglind et al., 2009).

Based on these lines of evidence, we hypothesized that, since adolescence is characterized by higher vulnerability to drugs of abuse, and given that protection toward such sensitivity is afforded by increased BDNF expression in the mPFC, repeated exposure to cocaine during development would lead to reduced expression of the neurotrophin and its associated signaling network in this brain region, thus explaining the higher vulnerability of adolescents to cocaine. To this end, we exposed male rats to repeated cocaine administration from post-natal day (PD) 28 to PD 42, a period of development that roughly approximates adolescence in humans (Collins and Izenwasser, 2004). Animals were then sacrificed after 3 d (PD 45) or 48 d (PD 90) of drug withdrawal to draw a dynamic picture of the effects produced by cocaine early and long after the end of adolescence.

Materials and methods

Experimental procedures

The adolescent rats used in this study were generated by mating Sprague–Dawley rats weighting 250 g (Italy), and were housed under standard conditions of temperature and humidity under artificial light (from 07:00 hours to 19:00 hours). No more than two male siblings were taken from each litter in order to reduce 'litter effects' (Chapman and Stern, 1978). Male rats were treated subcutaneously with cocaine (20 mg/kg d^{-1}) (MacFarlan-Smith, UK) or saline from PD 28 to PD 42, a period that roughly approximates adolescence in humans (Collins and Izenwasser, 2004). A group of rats, treated either with saline and cocaine, was sacrificed 3 d after the end of treatment (PD 45); another group of rats was left undisturbed in their home cages till adulthood and was sacrificed at PD 90. These time points were chosen in order to investigate BDNF and its associated network during early and long abstinence. Animals were always treated in the morning at the same time (12:00 hours), in order to avoid any stress due to unpredictability of the treatment. Both at PD 45 and PD 90, animals were sacrificed at 12:00 hours on the day of sacrifice. Although BDNF regulation is known to be dependent on environmental factors such as stress (Chourbaji et al., 2008, 2011, 2012), and stress is associated with non-contingent cocaine exposure (Twining et al., 2009), the long period of time between the treatment and the killing of the animals should avoid any potential bias due to stress-related effects. Following the sacrifice, the mPFC (defined as Cg1, Cg3, and IL subregions) corresponding to plates 5–9 of the atlas of Paxinos and Watson (Paxinos and Watson, 2005) was immediately dissected from 2 mm thick slices, frozen on dry ice and stored at −80°C.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., supplement 40, 18 Febbraio, 1992, Circolare No. 8, G.U., 14 Luglio, 1994) and international laws and policies (EEC Council Directive 86/609, OJL 358, 1, December 12, 1987; Guide for the Care & Use of Laboratory Animals, U.S. National Research Council, 1996).

RNA preparation and real-time polymerase chain reaction

RNA measures were taken in the same animals as the protein measures. Total RNA was isolated by single step guanidinium isothiocyanate/phenol extraction using PureZol RNA isolation reagent (Bio-Rad Laboratories, Italy) according to the manufacturer's instructions, and quantified by spectrophotometric analysis. Following total RNA extraction, the samples were processed for real-time reverse transcription polymerase chain reaction (real time RT-PCR) to assess mRNA levels, as previously described (Fumagalli et al., 2012). Briefly, an aliquot of each sample was treated with DNase to avoid DNA contamination. RNA was analysed by TaqMan qRT-PCR instrument (CFX384 real time system, Bio-Rad Laboratories) using the iScriptTM one-step RT-PCR kit for probes (Bio-Rad Laboratories). Each experimental group was composed of at least six rats. Samples were run in 384 well formats in triplicate as multiplexed reactions. Data were analysed with the comparative threshold cycle (ΔΔCt) method using 36B4, β-actin and 18S as reference genes (Barbon et al., 2010; Caffino et al., 2013). The primer efficiencies were experimentally set up for each couple of primers.

Probes and primers were purchased from Eurofins MWG-Operon. Their sequences are shown in Table 1. Thermal cycling was initiated with an incubation at 50°C for 10 min (RNA retrotranscription) and then at 95°C for 5 min (TaqMan polymerase activation). After this initial step, 39 cycles of PCR were performed. Each PCR cycle consisted of heating the samples at 95°C for 10 s to enable the melting process and then for 30 s at 60°C for the annealing and extension reaction.

Table 1. Sequences of forward and reverse primers and probes used in Real-Time PCR analyses

Gene	Forward primer	Reverse primer	Probe
BDNF	5′-AAGTCTGCATTACATTCCTCGA-3′	5′-GTTTTCTGAAAGAGGGACAGTTTAT-3′	5′-TGTGGTTTGTTGCCGTTGCCAAG-3′
BDNF exon IV	5′-CATATCGGCCACCAAAGACT-3′	5′-GTCATCACTCTTCTCACCTGG-3′	5′-TCTAGAACCTTGGGACCGGTCTT-3′
BDNF exon I	5′-GGGAGACGAGATTTTAAGACACT-3′	5′-GTCATCACTCTTCTCACCTGG-3′	5′-TTGTGGCTTTGCTGTCCTGGAGA-3′
BDNF exon VI	5′-CTGGCAGGCTTTGATGAGAC-3′	5′-GTCATCACTCTTCTCACCTGG-3′	5′-AGCTTTGTGTGGACCCTGAGTTCC-3′
BDNF exon IIb	5′-*AGTTGGCTTCCTAGCGGTGTA*-3′	5′-*GTCATCACTCTTCTCACCTGG*-3′	5′-*AATAGACTCTTGGCAAGCTCCGGTT*-3′
CaRF	5′-GAGATGCACACACCATTCCA-3′	5′-GTGTTGGCTCATTGGGTTCT-3′	5′-CAGCCATCCAGCTCTTGTTGAAGA-3′
Npas4	5′-GGAAGTTGCTATACCTGTCGG-3′	5′-GTCGTAAATACTGTCACCCTGG-3′	5′-CATAGAATGGCCCAGATGCTCGCT-3′
NF-kB	5′-CTACGAGACCTTCAAGAGCATC-3′	5′-GATGTTGAAAAGGCATAGGGC-3′	5′-AATGGACCAACTGAACCCCGGC-3′
Creb1a/b	5′-AGATTCTAGTGCCCAGCAAC-3′	5′-CTGTGCGAATCTGGTATGTTTG-3′	5′-TGTTCAAGCTGCCTCTGGTGATGT-3′
tPA	5′-CAAAATGAAGGGAGAGCTGTTG-3′	5′-TGTTGGTAAGTTGTCTGAGTCTG-3′	5′-CTGCTTTGTGGAGTGGCGTTCA-3′
β-actin	5′-CACTTTCTACAATGAGCTGCG-3′	5′-CTGGATGGCTACGTACATGG-3′	5′-TCTGGGTCATCTTTTCACGGTTGGC-3′
18S	5′-GTAACCCGTTGAACCCCATT-3′	5′-CCATCCAATCGGTAGTAGCG-3′	5′-TGCAATTATTCCCCATGAACGAGG-3′
36B4	5′-TTCCCACTGGCTGAAAAGGT-3′	5′-CGCAGCCGCAAATGC-3′	5′-AAGGCCTTCCTGGCCGATCCATC-3′

MicroRNA real-time qPCR

According to the manufacturer's instructions, 2 μl of RNA solution 5 ng/μl were reverse transcribed in 10 μl of reaction mix using the miRCURY LNA™ Universal RT microRNA PCR kit (#203300, Exiqon). cDNA was diluted 80× and assayed in real-time PCR according to the protocol for miRCURY LNA™ Universal RT microRNA PCR. SYBR green (SYBR® Green master mix, Exiqon, #203450) based RT-PCR was performed in CFX384 real-time system (Bio-Rad Laboratories) and LNA enhanced miRNA specific primers sets were used to quantify hsa-let7-d (#204124, Exiqon), hsa-miR-124 (#204319, Exiqon), hsa-miR-132 (#204129, Exiqon). Each reaction was performed in triplicate and data were analysed with the comparative threshold cycle (ΔΔCt) method using U6 snRNA (#203907, Exiqon) and RNU5G (#203908, Exiqon) as stably expressed reference genes.

Preparation of protein extracts and Western blot analyses

The mPFC was homogenized in a glass–glass potter using a cold buffer containing 0.32 M sucrose, 1 mM Hepes solution, 0.1 mM EGTA, 0.1 mM PMSF, pH=7.4, in the presence of a complete set of protease inhibitors and a phosphatase inhibitor cocktail. Crude synaptosomal fraction was prepared as previously described (Fumagalli et al., 2009a). The homogenized tissues were centrifuged at 1000 *g* for 10 min; the resulting supernatant was centrifuged at 9000 *g* for 15 min to obtain the pellet corresponding to the crude synaptosomal fraction, which was resuspended in a buffer containing 20 mM Hepes, 0.1 mM DTT, 0.1 mM EGTA, in the presence of a complete set of protease inhibitors and a phosphatase inhibitor cocktail. Total proteins were measured in the whole homogenate and in the crude synaptosomal fraction by the Bio-Rad Protein Assay, using bovine serum albumin as the calibration standard (Bio-Rad Laboratories, Italy). Equal amounts of protein were run under reducing conditions on the criterion TGX precast gels (Bio-Rad Laboratories, Italy) and then electrophoretically transferred onto polyvinylidene difluoride membranes (GE Healthcare, Italy).

Blots were blocked 1 h at room temperature with 10% non-fat dry milk in TBS +0.1% Tween-20 buffer, incubated with antibodies against the phosphorylated forms of the proteins and then stripped and reprobed with the antibodies against corresponding total proteins.

The conditions of the primary antibodies were the following: proBDNF (1:2000, GeneTex, USA); mBDNF (1:500, Santa Cruz Biotechnology, USA); anti total trkB (1:750, Santa Cruz Biotechnology, USA); anti phopsho-ERK2 T185/187 (1:1000, Cell Signaling Technology, USA); anti total ERK2 (1:5000, Santa Cruz Biotechnology, USA); anti phopsho-Akt S473 (1:1000, Cell Signaling Technology, USA); anti total Akt (1:1000, Cell Signaling Technology, USA); anti phopsho-mTOR S2448 (1:1000,

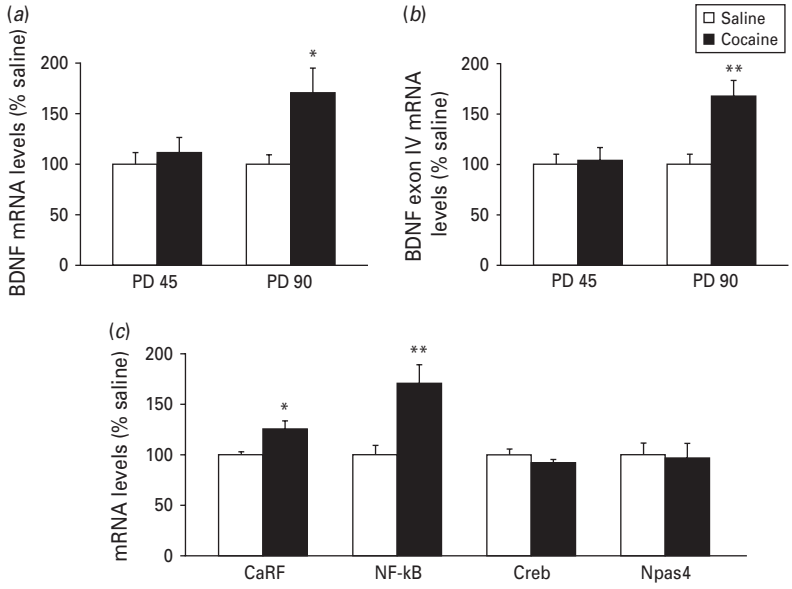

Fig. 1. Developmental exposure to cocaine alters the transcriptional regulation of BDNF: molecular mechanisms. Panel (*a*) and (*b*) show total and exon IV BDNF mRNA levels, respectively, in the mPFC of PD 45 and PD 90 rats following repeated exposure to cocaine during adolescence. Panel (*c*) shows the mRNA levels of several transcription factors involved in the regulation of exon IV mRNA levels in the mPFC of PD 90 rats. The results, expressed as % of saline-treated rats, represent the mean±S.E.M. of six samples; *$p<0.05$ and **$p<0.01$ *vs.* saline-treated rats.

Cell Signaling Technology, USA); anti total mTOR (1:1000, Cell Signaling Technology, USA); anti phopsho-S6 ribosomal protein S240/244 (1:1000, Cell Signaling Technology, USA); anti total S6 ribosomal protein (1:1000, Cell Signaling Technology, USA); anti total GluA1 (1:2000, Upstate, USA); anti Arc (1:500, BD Transduction Laboratories, USA) and anti *β*-Actin (1:10000, Sigma-Aldrich, Italy). Results were standardized using *β*-actin as the control protein, which was detected by evaluating the band density at 43 kDa. Immunocomplexes were visualized by chemiluminescence using the Chemidoc MP Imaging System (Bio-Rad Laboratories).

Statistical analysis

Data were collected on individual animals (independent determinations) and are presented as means and standard errors. The effects produced by repeated cocaine treatment were analysed by an unpaired Student's *t* test. Statistical significance was assumed at $p<0.05$.

Results

Figure 1(*a*) shows that repeated exposure to cocaine during adolescence increases BDNF mRNA levels in the mPFC of PD 90 (+71%, $p<0.05$), but not PD 45 rats (+11%, $p>0.05$). The analysis of the different BDNF isoforms at the 5′-UTR revealed that such increase could be ascribed to BDNF exon IV (+68%, $p<0.01$) (Fig. 1(*b*)), i.e. the most abundant isoform, sensitive to neuronal activity (Pruunsild et al., 2011). No changes were instead observed in the mRNA levels of other major transcripts expressed in the prefrontal cortex (exon I, exon II, exon VI) (data not shown). The analysis of the transcription factors involved in the modulation of BDNF exon IV revealed a selective activation of nuclear factor B (NF-kB) (+70%, $p<0.01$) and calcium responsive factor (CaRF) (+25%, $p<0.05$) in the mPFC of PD 90 rats treated with cocaine during adolescence (Fig. 1(*c*)). No changes were instead observed for two other transcription factors regulating exon IV (Fig. 1(*c*)), namely cAMP responsive element binding protein (Creb) (−8%, $p>0.05$) and neuronal Per-Arnt-Sim domain protein 4 (Npas4) (−3%, $p>0.05$).

The transcriptional changes of BDNF after repeated exposure to cocaine were paralleled by significant alterations in its protein levels, as shown in Fig. 2. Developmental exposure to cocaine increased both proBDNF and mBDNF levels in the homogenate of PD 90 rats (proBDNF: +20%, $p<0.05$; mBDNF: +27%, $p<0.01$) (Fig. 2*b*) but not in PD 45 rats (proBDNF: −7%, $p>0.05$; mBDNF: −7%, $p>0.05$) (Fig. 2(*a*)), suggesting an increased synthesis and processing of BDNF. Given that a significant effect on BDNF was observed only at PD 90, from now on we focused our attention on this time point.

The increased expression of proBDNF may be sustained by alterations in the expression of some miRNAs regulating BDNF expression. In particular, we focused our attention on let7-d, miR-124 and miR-132, three different microRNAs that regulate, among the others, cocaine-induced BDNF expression (Chandrasekar and Dreyer, 2009). Indeed, we observed a significant decrease of these miRNAs (let7-d: −81%, $p<0.001$; miR-124: −66%,

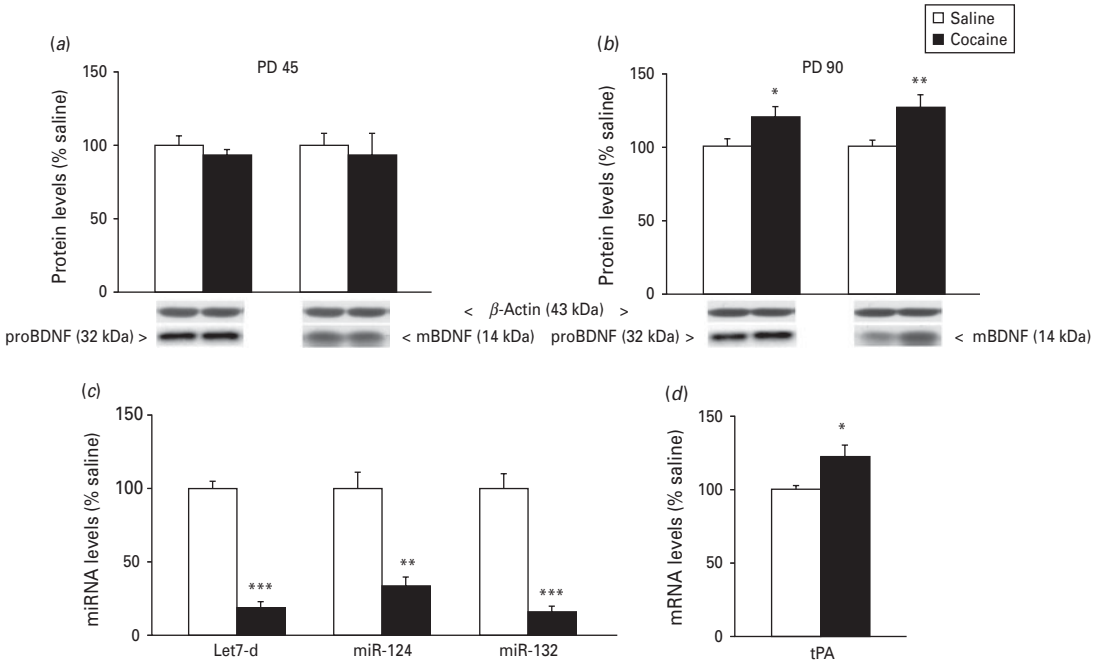

Fig. 2. Developmental exposure to cocaine alters the translational regulation of BDNF: molecular mechanisms. Panel (*a*) and (*b*) show the levels of precursor BDNF (proBDNF) and mature BDNF (mBDNF), respectively, in the mPFC from PD 45 and PD 90 rats following repeated exposure to cocaine during adolescence. Representative Western blot bands of pro- and mBDNF are shown below the graphs shown in panel (*a*) and (*b*). Panel (*c*) shows the modulation of several microRNA (miRNAs) implicated in the regulation of BDNF following exposure to cocaine. Panel (*d*) shows the mRNA levels of tissue plasminogen activator (tPA), one of the extracellular proteases that govern the proteolytic conversion of proBDNF into mBDNF. The results, expressed as % of saline-treated rats, represent the mean±S.E.M. of six samples; *$p<0.05$, **$p<0.01$ and ***$p<0.001$ *vs.* saline-treated rats.

$p<0.01$ and miR-132: −84%, $p<0.001$), which may contribute to the increased expression of proBDNF (Fig. 2(*c*)).

Furthermore, we found that the mRNA levels of tissue plasminogen activator (tPA), one of the extracellular proteases converting proBDNF into mBDNF (Pang et al., 2004), were significantly increased in the mPFC of cocaine-treated rats at PD 90 (+22%, $p<0.05$) (Fig. 2(*d*)).

We also found an increased trkB expression in the crude synaptosomal fraction of the mPFC from cocaine-withdrawn rats (+35%, $p<0.05$) with no changes in the homogenate (+9%, $p>0.05$) (Fig. 3(*a*)), suggesting an increased trafficking of the receptor toward the membrane. Moreover, we found that prolonged abstinence from developmental cocaine induced a selective activation of Akt (pAkt S473: +39%, $p<0.05$; Akt: +12%, $p>0.05$) but not ERK2 (pERK2 T185/187: −10%, $p>0.05$; ERK2: −3%, $p>0.05$) (Fig. 3(*b*)). Akt activation is linked to the pathway of mTOR and S6K that, accordingly, were examined under our experimental conditions. We found that, in the mPFC of cocaine-withdrawn rats, the phosphorylation of mTOR is enhanced (pmTOR S2448: +30%, $p<0.05$; mTOR: −9%, $p>0.05$) and so is the phosphorylation of S6K (pS6K S240/244: +96%, $p<0.001$; pS6K: +22%, $p>0.05$) (Fig. 3(*c*)), suggesting an overall activation of the translational machinery as a consequence of prolonged abstinence.

Among the different targets of S6, we decided to investigate the expression of the immediate early gene Arc, an effector early gene with a multifaceted role in brain plasticity. In fact, changes in Arc expression may represent changes in neuronal activity but also may impact synaptic plasticity, by altering, for example, the surface expression of glutamate AMPA receptors (Chowdhury et al., 2006; Rial Verde et al., 2006). Interestingly, abstinence-induced S6 activation resulted in increased expression of Arc in the cortical crude synaptosomal fraction of cocaine-withdrawn rats (+24%, $p<0.05$) (Fig. 3(*d*)). Such increase resulted in reduced membrane AMPA receptor expression (−22%, $p<0.05$) (Fig. 3(*e*)).

Discussion

Our data are the first to show that abstinence from adolescent exposure to cocaine increases BDNF and its signaling network in the medial prefrontal cortex (mPFC) of adult rats. These results provide novel mechanisms governing abstinence-induced changes in BDNF and its related signaling, suggesting that withdrawal from cocaine exposure during adolescence may impact adult brain homeostasis.

In particular, BDNF expression is increased as a function of abstinence duration, since its expression is not altered 3 d after the end of treatment, but only 48 d later. Notably, prolonged abstinence from developmental cocaine exposure selectively up-regulates BDNF exon IV, which represents the major activity-dependent transcript

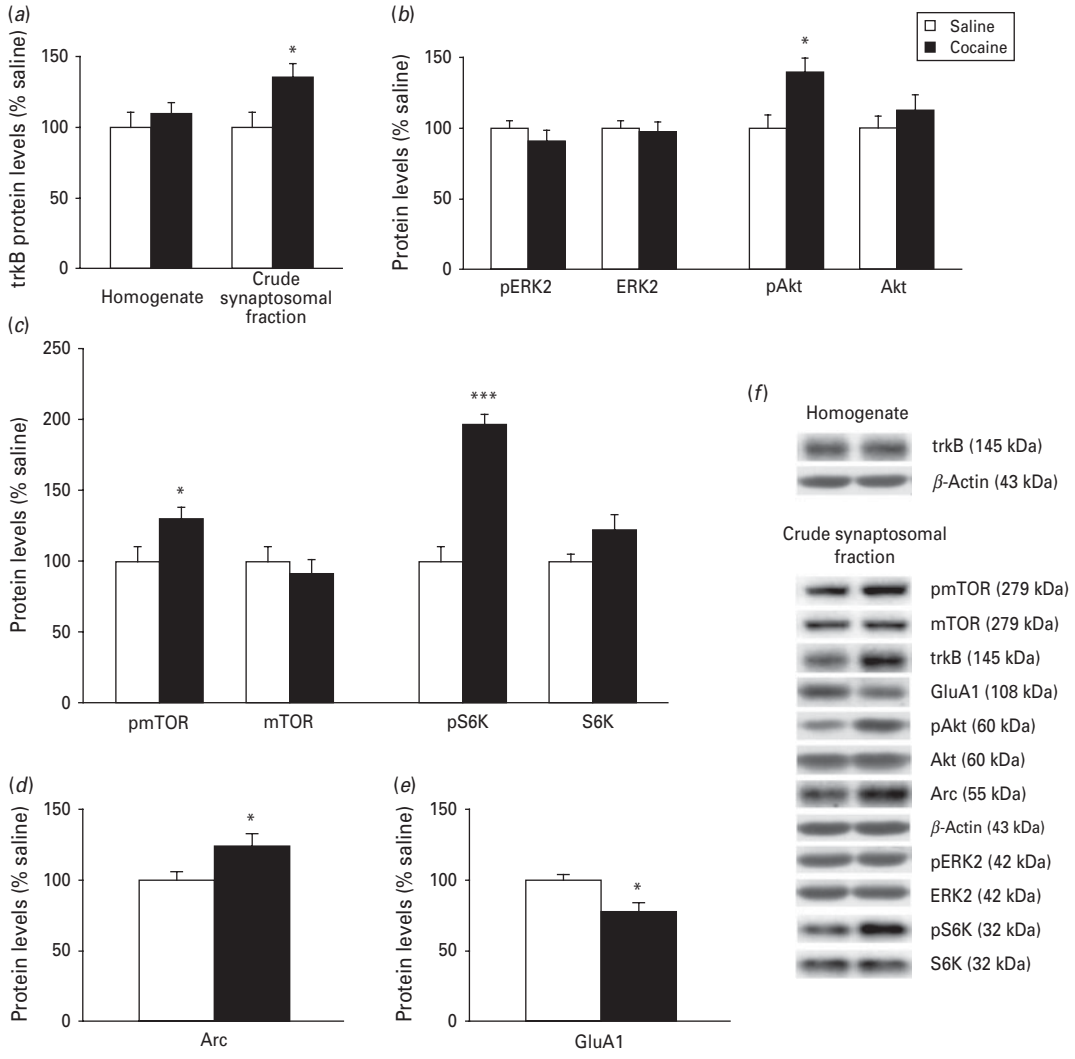

Fig. 3. Developmental exposure to cocaine alters BDNF-induced intracellular pathways and neuroplastic markers. Panel (*a*) shows the expression of the high affinity BDNF receptor trkB following repeated exposure to cocaine in the mPFC of PD 90 rats in the whole homogenate and crude synaptosomal fraction. Panel (*b*) shows the phosphorylation and expression of ERK 2 and Akt in the mPFC of PD 90 rats in the crude synaptosomal fraction. Panel (*c*) shows the activation of the Akt-dependent pathway (mTOR and S6K) in the crude synaptosomal fraction. Panel (*d*) shows the expression of the immediate early gene Arc whereas panel (*e*) shows the expression of the main AMPA subunit of glutamate receptors, i.e. GluA1, in the crude synaptosomal fraction. Representative Western blot bands are shown in panel (*f*). The results, expressed as % of saline-treated rats, represent the mean±S.E.M. of six samples; $*p<0.05$ and $***p<0.001$ *vs.* saline-treated rats.

of the neurotrophin. This presumably occurs through the activation of transcription factors such as NF-kB and CaRF that are known to regulate BDNF exon IV.

Changes in BDNF mRNA levels were accompanied by an up-regulation of BDNF protein levels, suggesting that abstinence from adolescent cocaine exposure regulates also the translation of the neurotrophin. Abstinence-induced increase of BDNF protein levels occurs through at least two distinct mechanisms. First, abstinence from repeated exposure to cocaine during adolescence causes a profound down-regulation of some miRNAs that are known to control BDNF expression (Chandrasekar and Dreyer, 2009), an effect that may very well contribute to the long-lasting proBDNF up-regulation herein observed. Second, the mRNA levels of tPA, the enzyme responsible of the cleavage of proBDNF into mBDNF, is increased in the mPFC of PD 90 rats exposed to cocaine during adolescence. The up-regulation of the extracellular proteases tPA is indicative of increased processing that leads to higher levels of mBDNF.

The increase of BDNF expression represents the first step of a series of dynamic changes that lead to an over-activity of BDNF-dependent pathways. In fact, we found a parallel up-regulation of its high affinity receptor trkB that might be considered an index of activation upon neurotrophin release (Saarelainen et al., 2003). TrkB enhancement, in turn, resulted in a selective activation of the Akt-dependent pathway that subsequently activated both mTOR and S6 phosphorylation (Gong et al., 2006), a canonical pathway known to be activated by

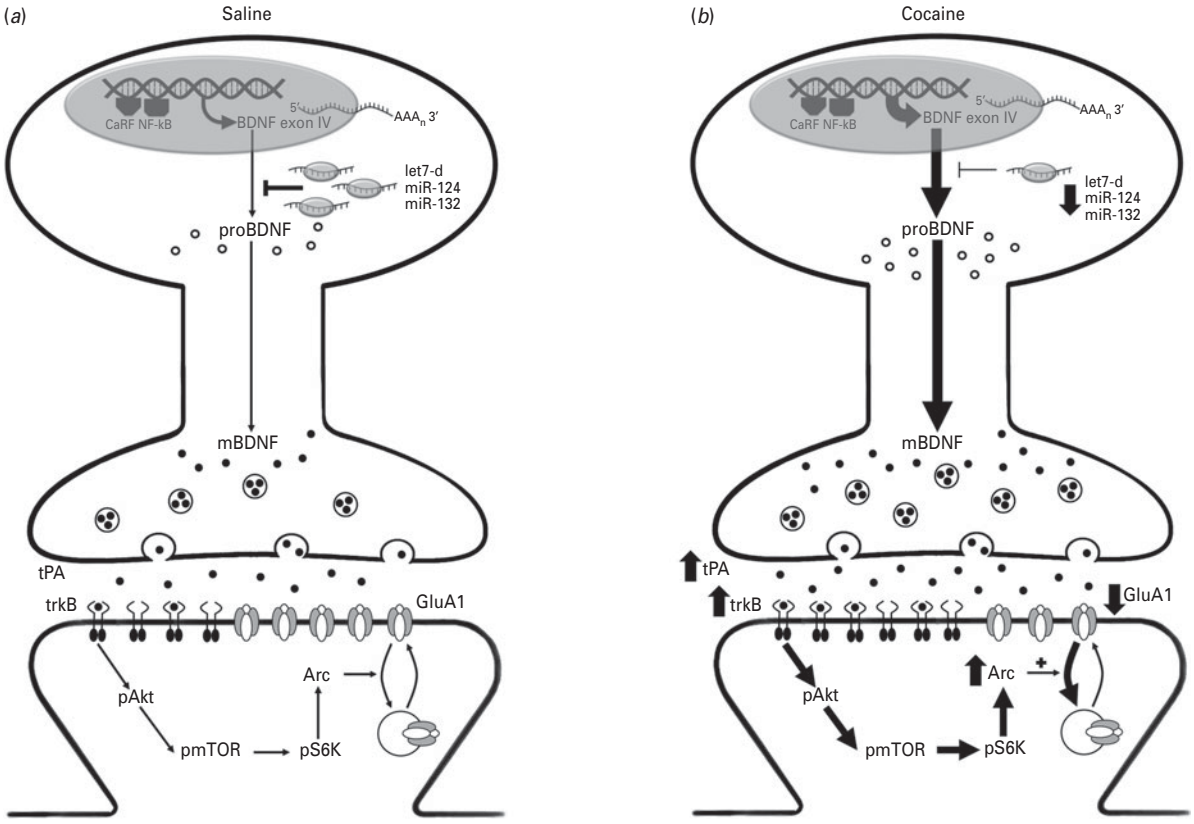

Fig. 4. Schematic representation of the changes of BDNF and its associated network set in motion by the developmental exposure to cocaine (panel *b*): comparison with rats exposed to saline (panel *a*).

BDNF (Swiech et al., 2008) that promotes mRNA translation (Hoeffer and Klann, 2010). These results suggest that prolonged abstinence from cocaine exposure during adolescence permanently alters the physiological regulation of the translational machinery. Interestingly, dysregulation of this pathway has been observed in the brain of cocaine addicts (Alvaro-Bartolome et al., 2011).

The widespread impact on neuroplasticity exerted by the abstinence from developmental exposure to cocaine is further highlighted by the increased expression of Arc. Arc is a critical crossroad of various signals that converge into the stabilization of synaptic inputs (Bramham et al., 2010) and it has been implicated in the acute or chronic action of cocaine (Fumagalli et al., 2006, 2009b; Caffino et al., 2011; Hearing et al., 2011) as well as in the relapse to cocaine seeking (Hearing et al., 2008). Arc is a target of the mTOR–S6 kinase pathway (Panja and Bramham, 2013) and, accordingly, we observed increased Arc expression in the mPFC of PD 90 rats, thus identifying Arc as a marker of developmental cocaine-induced long-term adaptations. Interestingly, recent evidence has shown a crucial role for Arc in regulating AMPA-type glutamate receptor endocytosis (Chowdhury et al., 2006; Rial Verde et al., 2006). Accordingly, the levels of GluA1, the main AMPA subunit, are reduced in the crude synaptosomal fraction of mPFC from PD 90 cocaine-withdrawn rats, suggesting that long-term abstinence from developmental cocaine exposure indirectly influences glutamate neurotransmission, presumably through changes in the BDNF–Arc pathway.

To the best of our knowledge, this is the first evidence showing that abstinence from repeated exposure to cocaine during adolescence alters the expression of BDNF and its associated network in adulthood, thus ruling out the possibility that the increased vulnerability of adolescents to drugs of abuse might rely upon the reduction of BDNF levels. Our data are in conflict with recent published evidence showing reduced BDNF expression following early, but not long, abstinence from repeated cocaine treatment in adulthood (McGinty et al., 2010), an effect that seems to occur via alteration of the MAP kinase pathway (Whitfield et al., 2011). The most parsimonious explanation for this discrepancy is that the timing of cocaine exposure (development *vs.* adulthood) dictates the profile of BDNF expression. Intriguingly, the effect caused by the abstinence from cocaine exposure during adolescence seems to depend upon the activation of a pathway different from that activated by the adult treatment (i.e. PI3 kinase instead of MAP kinase), since we found activation of Akt but not ERK 2. Our results are in line with the hypothesis that enhanced BDNF expression might represent an adaptive, defensive strategy to oppose cocaine-seeking and might be interpreted as a compensatory reaction in an attempt, perhaps, to

normalize drug-induced alterations in glutamate transmission in the NAc (Berglind et al., 2009). Interestingly, although there is no evidence of cocaine-induced changes in the mTOR pathway in the mPFC, it has been demonstrated that, in the NAc, such a pathway regulates cocaine seeking (Wang et al., 2010). In analogy with the protective effect evoked by BDNF infusion in the mPFC (Berglind et al., 2007), we may speculate that the herein shown increase of the mTOR pathway may prevent cocaine seeking.

Conclusions

Long-term abstinence from repeated exposure to cocaine during adolescence causes a significant up-regulation of the neurotrophin BDNF that occurs through different mechanisms both at transcriptional and translational level. Such increase promotes increased phosphorylation of Akt, through the high affinity BDNF receptor trkB that culminates in the activation of the mTOR-S6 kinase pathway, indicating that mRNA translation and long-lasting synaptic plasticity is altered in the mPFC of cocaine-withdrawn adolescent rats. The effects of the abstinence from the developmental exposure to cocaine, however, go beyond the alteration of the BDNF system, resulting in translational changes of Arc and GluA1 expression that reveal a global effect on mPFC neuroplasticity (Fig. 4).

Taken together, these data show that abstinence from repeated exposure to cocaine during adolescence sets in motion disparate mechanisms that affect a wide variety of targets involved in brain plasticity. These findings indicate that interfering with the correct development of the mPFC by repeated exposure to cocaine dysregulates the BDNF system and its associated network, and reveal novel mechanisms that underlie the prolonged abstinence from early-in-life exposure to cocaine. This previously unappreciated and highly dynamic way of regulating cortical neuroplasticity by early delivery of, and long-term abstinence from, adolescent cocaine may provide novel potential targets for the treatment of psychostimulant abuse.

Acknowledgment

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Conflict of Interest

None.

Note

These results were presented in a symposium at the 24th Biennial Joint Meeting of the International Society for Neurochemistry and American Society for Neurochemistry, held in Cancun, Mexico, 20–24 April 2013. The symposium was entitled 'Cocaine-induced BDNF Plasticity in the Developing and Mature Brain', co-chairs: G. Sadri-Vakili and F. Fumagalli. The abstract sent for the speech of Professor Fumagalli was entitled 'Adolescent exposure to cocaine dynamically alters BDNF expression in the rat brain'.

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