Research Paper

Neurotrophic factors in women with crack cocaine dependence during early abstinence: the role of early life stress

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Background: Neurotrophic factors have been investigated in the pathophysiology of alcohol and drug dependence and have been related to early life stress driving developmental programming of neuroendocrine systems. **Methods:** We conducted a follow-up study that aimed to assess the plasma levels of glial cell line–derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT3) and neurotrophin-4/5 (NT4/5) in crack users during 3 weeks of early abstinence in comparison with healthy controls. We performed a comprehensive clinical assessment in female inpatients with crack cocaine dependence (separated into 2 groups: participants with (CSA+) and without (CSA–) a history of childhood sexual abuse) and a group of nonuser control participants. **Results:** Our sample included 104 women with crack cocaine dependence and 22 controls; of the women who used crack cocaine, 22 had a history of childhood sexual abuse and 82 did not. The GDNF plasma levels in the CSA+ group increased dramatically during 3 weeks of detoxification. In contrast, those in the CSA– group showed lower and stable levels of GDNF under the same conditions. Compared with the control group, BDNF plasma levels remained elevated and NGF levels were reduced during early abstinence. We found no differences in NT3 and NT4/5 between the patients and controls. However, within-group analyses showed that the CSA+ group exhibited higher levels of NT4/5 than the CSA– group at the end of detoxification. **Limitations:** Some of the participants were using neuroleptics, mood stabilizers or antidepressants; our sample included only women; memory bias could not be controlled; and we did not investigate the possible confounding effects of other forms of stress during childhood. **Conclusion:** This study supports the association between early life stress and peripheral neurotrophic factor levels in crack cocaine users. During early abstinence, plasmastic GDNF and NT4/5 were the only factors to show changes associated with a history of childhood sexual abuse.

Introduction

Neurotrophic factors (NFs) have been investigated in the pathophysiology of alcohol and drug dependence.[1–4] Because preclinical evidence has suggested a potential role of these endogenous peptides in mediating the behavioural effects related to substance administration[5] and drug-induced neuroadaptation,[6] recent studies have explored neurotrophin expression in humans[4,7,8] to better comprehend the neurobiological and circuit underpinnings of addiction.

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Neurotrophic factors play a central role in brain development and are known for their role in mediating neuronal plasticity and neuronal growth in the central and peripheral nervous systems. In addition, in adulthood, NFs are important for synaptic plasticity and dendritic growth and are essential for the consolidation of long-term memory.[9] The NF family includes neurotrophins, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT3) and neurotrophin-4/5 (NT4/5), and glia family ligands, such as glial cell line–derived neurotrophic factor (GDNF).[10] In addition to neurons, NFs are produced by distinct cell types, including immune cells, adipocytes and endocrine and endothelial cells, thus being in a position to affect and integrate neural, immune and endocrine system functioning.[11] Substantial evidence has shown that NFs also interact with neurotransmitter systems.[12,13] Their effects on dopaminergic neuronal function in particular may be a critical path for drug addiction because the dopamine (DA) system is a key component of the brain circuitry of motivation and reward.[14–16]

Preclinical investigations have demonstrated that cocaine administration can increase the levels of BDNF in rodents.[17,18] Furthermore, recent studies have shown a significant increase in BDNF levels over the first days of cocaine abstinence in both preclinical and clinical models, which is most likely due to compensatory mechanisms activated in response to the neurotoxic effects of chronic cocaine exposure.[4,19] Levels of BDNF following cocaine detoxification also have predictive value for the loss of control measures affecting craving and relapse.[2,8] However, contrary to the BDNF findings, data from human and animal studies have shown decreased NGF levels after both heroin and cocaine withdrawal.[3,6] These data indicate that NF expression may be impacted differently within drug abstinence periods.

Childhood maltreatment is a robust risk factor for drug addiction, and a variety of studies have shown a biological embedding of early life experiences.[20–22] Data from rodents and nonhuman primates suggest that changes in plasma levels of neurotrophins might function as peripheral markers of early adversity, being differentially affected by changes in the rearing environment.[23] Specifically, increased peripheral levels of NGF and overall reduced BDNF levels have been observed in monkeys experiencing maternal separation.[23] Accordingly, some authors have suggested that NF changes could be related to early life stress (e.g., exposure to childhood sexual abuse), which drives a developmental programming of neuroendocrine systems implicated in vulnerability to later mood disorders[24] or drug addiction.[25]

To our knowledge, this is the first investigation that includes a comprehensive assessment of NFs in women with crack cocaine dependence during the early stages of abstinence. Most clinical studies have investigated only the expression of specific neurotrophins, particularly BDNF and NGF, in relation to ongoing drug use, and there are very few reports regarding the consequences of early life stress exposure on NF expression. Therefore the aim of our study was to assess plasma levels of BDNF, NGF, NT3, NT4/5 and GDNF during 3 weeks of early abstinence in women with crack cocaine dependence with and without a history of childhood sexual abuse in comparison to healthy controls.

Methods

Participants

We conducted a follow-up study including a convenience sample of women with crack cocaine dependence recruited after admission to a 3-week, locked detoxification treatment facility for women with drug and alcohol dependence. We further separated these women into 2 groups based on the presence (CSA+ group) or absence (CSA– group) of a history of childhood sexual abuse.

Inpatients had no access to alcohol, cigarettes or drugs during treatment. In addition, all patients had a prescribed symptomatic cocaine detoxification protocol that included neuroleptics, antidepressants and mood stabilizers. The inclusion criteria for were as follows: age 18–55 years; diagnosis of a substance use disorder, physiologic dependence of crack cocaine type; absence of self-reported comorbid psychotic syndromes; a minimum of 4 years of formal education; and absence of severe medical conditions. We excluded individuals who were found to have previously undetected psychotic symptoms after a psychiatric interview; those who were found to have neurologic diseases, infectious or metabolic disorders after medical assessment; and those with any severe cognitive deficits that resulted in an altered state of consciousness or agitation and use of benzodiazepines.

In addition, we also recruited unmedicated, healthy, age-matched control participants from among 90 volunteers selected by convenience sampling. We excluded from the control group any individuals with past or current axis I disorders, history of childhood maltreatment, severe or unstable clinical illness, neurologic disorder, or any psychoactive substance use in the 30 days preceding the study.

Clinical assessment

We used the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID I)[26] to determine the participants' diagnoses and comorbid syndromes. History of childhood sexual abuse was assessed using the Childhood Trauma Questionnaire (CTQ)[27] and was classified from moderate to extreme according to the cutoff point postulated by Bernstein and colleagues[28] for adult women. We evaluated the severity of depressive symptoms using the Beck Depression Inventory (BDI-II),[29] and we rated the severity of abstinence symptoms using the Cocaine Selective Severity Assessment (CCSA).[30] The BDI-II and CSSA were administered after 4 days of detoxification and then repeated once a week for the subsequent 2 weeks (for a total of 3 assessments).

The Addiction Severity Index (ASI-6)[31,32] is a semistructured interview that was administered to assess multiple dimensions of the severity of substance use problems and the pattern of substance use, including the frequency, quantity and severity of drug use for multiple substances. The ASI-6 also assesses the

severity of problems in the following domains: health, legal, occupational, sociofamilial, psychiatric and use of alcohol. An expert psychiatrist (J.C.P.) and 3 well-trained psychologists (T.W.V. and S.G.T., as well as I.A.F. [not among the authors]) administered all of the clinical assessments. The ethical committee of all the enrolled institutions approved our study protocol, and all of the participants provided written informed consent.

Plasma neurotrophic factor levels

Blood was collected in ethylenediaminetetraacetic acid tubes from 11 am to 11:30 am, 3 hours after the participants' last meal. Participants with crack dependence had their plasma collected on the fourth, eleventh and eighteenth day of the detoxification period. We started collecting blood 4 days after admission since acute intoxication could be a problem for many participants in the first 3 days of treatment. The blood was separated within 30 minutes, and the supernatant was stored at –80°C for up to 6 months. We measured the plasma levels of BDNF, NGF, GDNF, NT3 and NT4/5 using a commercially available immunoassay enzyme-linked immunosorbent assay kit (R&D Systems) as per the manufacturer's instructions. All samples were assayed in duplicate. All plasma NF levels are expressed as picograms per millilitre. The detection limits were 5 pg/mL for BDNF and 10 pg/mL for NGF, GDNF, NT3 and NT4/5.

Statistical analysis

The distributions of all variables were tested for normality using the Shapiro–Wilks test. The demographic, clinical and psychosocial variables were examined using χ^2 tests or analysis of variance (ANOVA), as appropriate. The distributions of the ASI-6 variables and subscales failed tests of normality; thus, they were analyzed using the Wilcoxon Mann Whitney test. We examined the BDI-II and CSSA variables using unpaired *t* tests. Regarding NGF, NT3, NT4/5 and GDNF, the data were log-transformed to improve normality. Analyses of covariance (ANCOVA), including group as a between-subjects factor and covarying for age and body mass index (BMI) were used to analyze single parameters in each point of assessment.

To examine NF differences between the CSA+ and CSA– groups during follow-up, we performed univariate repeated-measures ANCOVAs, covarying for age, BMI and depression severity change [BDI-II scores: ((time3 – time1) ÷ time1) × 100]. Bonferroni adjustments were used for pairwise comparisons with an α of 0.05. We selected the covariates based on evidence that plasma levels of BDNF decrease significantly with increasing age or weight[33] and that depression score changes are correlated to changes in BDNF levels.[34] We performed all statistical analyses using SPSS version 20.0.

Results

Our sample included 104 women (22 in the CSA+ group and 82 in the CSA– group) with crack cocaine dependence and 20 controls. The demographic, clinical and psychosocial characteristics of participants are summarized in Table 1 and Table 2. All groups were similar in age, BMI and menstrual cycle stage. There was no significant effect of menstrual cycle stage on any NF levels in any group. There were no significant effects of a comorbid psychiatric diagnosis on NF levels for either group of

Table 1: Demographic, clinical and psychosocial characteristics of the groups

Characteristic	Group; no. (%)*			Statistic	*p* value
	CSA+, *n* = 22	CSA–, *n* = 82	Control, *n* = 20		
Age, mean ± SD yr	31.8 ± 6.2	28.1 ± 7.6	29.5 ± 8.8	$F_{2,120} = 2.12$	0.12
Menstrual cycle				$\chi^2_2 = 0.42$	0.80
Follicular phase	13 (59.1)	47 (57.3)	10 (50)	—	—
Luteal phase	9 (40.9)	35 (42.6)	10 (50)	—	—
Comorbidities					
Depressive episode	6 (27.2)	13 (15.8)	—	$\chi^2_1 = 1.35$	0.24
Bipolar disorder	1 (4.5)	11 (13.4)	—	$\chi^2_1 = 1.16$	0.28
Posttraumatic stress disorder	5 (22.7)	7 (8.5)	—	$\chi^2_1 = 2.98$	0.08
Other axis I disorder	4 (18.1)	8 (9.7)	—	$\chi^2_1 = 1.53$	0.21
Medication					
Mood stabilizers	10 (45.5)	44 (53.7)	—	$\chi^2_1 = 0.46$	0.49
Neuroleptics	10 (45.5)	48 (58.5)	—	$\chi^2_1 = 1.21$	0.27
Antidepressants	1 (4.5)	7 (8.5)	—	$\chi^2_1 = 0.38$	0.53
CTQ–CSA, mean ± SD	18.31 ± 5.25	6.08 ± 2.52	—	$U = 98$†	< 0.001
BMI, mean ± SD					
Baseline	22.8 ± 3.1	22.3 ± 3.5	23.7 ± 3.2	$F_{2,121} = 1.40$	0.24
11 d	23.7 ± 3.2	23.1 ± 3.7	—	$F_{2,121} = 0.43$‡	0.65
18 d	24.3 ± 3.5	23.9 ± 3.4	—	$F_{2,121} = 0.14$‡	0.86

BMI = Body Mass Index; CSA+ = crack cocaine dependence with a history of childhood sexual abuse; CSA– = crack cocaine dependence without a history of childhood sexual abuse; CTQ = Childhood Trauma Questionnaire; SD = standard deviation.
*Unless otherwise indicated.
†*U* = Wilcoxon Mann Whitney test.
‡Phase of menstrual cycle during first week of hospitalization.

women with crack cocain dependence. No significant associations were found between pharmacotherapy use and GDNF, NGF and NT4/5 measures (all $p > 0.05$, assessed by t test). However, the use of neuroleptics and antidepressants was independently associated with higher levels of BDNF on day 18 and with higher levels of NT3 on days 4, 11 and 18 (all $p < 0.05$) in women with crack cocaine dependence, regardless of CSA subgroup. Nevertheless, both CSA groups were homogeneous regarding medication and comorbid diagnoses.

The CSA+ group had significantly greater psychiatric impairment according to the AIS-6 subscale scores and also reported significantly more hospital admissions owing to relapse in the 6 months preceding the study than the CSA– group. Conversely, the pattern of crack cocaine use, age at onset of drug use and number of days of withdrawal before being admitted for treatment were similar between these 2 groups. The patterns of marijuana and alcohol use were also similar. In addition, the groups were very similar regarding severity of depressive and withdrawal symptoms during early abstinence, despite the marginally increased effect that could be perceived in the CSA+ group after 18 days of detoxification. Altogether these findings suggest that women in the CSA+ group may have been more vulnerable to the impact of crack consumption but that the important clinical variables that could impact NF levels were controlled.

To evaluate the role of childhood sexual abuse in women with crack cocaine dependence, we used a repeated-measures multivariate general linear model adjusted for age and BMI for each NF. These analyses revealed a statistically significant group × time interaction effect for GDNF ($F_{2, 130} = 8.46$, $p = 0.001$; Mauchly's W = 0.88, Greenhouse–Geisser-corrected, $p = 0.001$) and NT4/5 levels ($F_{2, 138} = 4.44$, $p = 0.013$; Mauchly's W = 0.93, Greenhouse–Geisser-corrected, $p = 0.90$) during the detoxification period. Furthermore, we identified a significant general effect of time for NGF levels ($F_{2,65} = 3.76$, $p = 0.026$; Mauchly W = 0.93, $p = 0.10$) during detoxification, regardless of history of childhood sexual abuse; however, this result did not remain significant after pairwise comparisons. There were no significant effects of group or time on BDNF and NT3 levels in this analysis (Fig. 1).

To investigate whether the GDNF and NT4/5 effects are best predicted by severity of crack cocaine addiction, by childhood sexual abuse or by medication, we performed a stepwise multiple linear regression, including sexual abuse score (CTQ), severity of drug addiction (ASI-6), use of medication, age and BMI, predicting GDNF and NT4/5 plasma levels after 3 weeks of detoxification. Only childhood sexual abuse (B = 0.27, $p = 0.012$) and BMI (B = 0.23; $p = 0.031$) were included in the equation predicting GDNF after treatment [$R = 0.36$, $R^2 = 0.13$; $F_{1,74} = 4.81$, $p = 0.031$].

Table 2: Clinical characteristics of groups

Characteristic	Group; mean ± SD*		Statistic*	p value
	CSA+, $n = 22$	CSA–, $n = 82$		
ASI-6				
Drugs	60.7 ± 8.8	63.2 ± 10.1	$U = 790$	0.36
Family/child	67.5 ± 8.3	66.9 ± 8.9	$U = 861$	0.74
Alcohol	50.8 ± 9.7	50.4 ± 9.7	$U = 871$	0.80
Psychiatric diagnosis	59.0 ± 6.0	56.4 ± 5.3	$U = 618$	0.017
Medical	47.8 ± 7.7	47.0 ± 6.7	$U = 807$	0.45
Legal	58.7 ± 9.5	62.4 ± 8.1	$U = 677$	0.07
Employment	44.0 ± 6.9	44.0 ± 8.2	$U = 899$	0.98
Family social support	46.9 ± 12.4	45.6 ± 11.8	$U = 834$	0.58
Family social problem	56.4 ± 9.0	56.4 ± 9.2	$U = 898$	0.97
Prior hospital admissions in last 6 mo owing to relapse, no. (%)	10 (45.5)	15 (19.5)†	$\chi^2_1 = 6.11$	0.013
Age at first crack cocaine use, yr	19.2 ± 6.4	18.5 ± 5.2	$U = 657$	0.92
Duration, yr, of crack use ≥ 3 times/wk	5.5 ± 5.3	4.6 ± 3.4	$U = 703$	0.99
Days of consumption in last mo	15.6 ± 13.5	16.2 ± 13.2	$U = 614$	0.79
Withdrawal prior to admission, d	3.9 ± 7.3	2.3 ± 3.8	$U = 750$	0.85
Alcohol use > 50 times, no. (%)	11 (50)	37 (45.1)	$\chi^2_1 = 0.39$	0.53
Powder cocaine use > 50 times, no. (%)	10 (45.4)	58 (70.7)	$\chi^2_1 = 3.44$	0.06
Marijuana use > 50 times, no. (%)	22 (100)	82 (100)	—	—
Beck Depression Inventory				
4 d	33.5 ± 15.5	29.0 ± 14.6	$t_{102} = -1.28$	0.20
11 d	24.2 ± 14.2	20.7 ± 13.5	$t_{102} = -1.07$	0.28
18 d	18.7 ± 12.3	17.4 ± 11.3	$t_{102} = -1.28$	0.63
Cocaine Selective Severity Assessment				
4 d	60.9 ± 17.6	55.6 ± 17.4	$t_{101} = -1.22$	0.22
11 d	53.4 ± 30.0	47.6 ± 18.5	$t_{101} = -1.11$	0.26
18 d	53.0 ± 20.3	45.3 ± 16.4	$t_{100} = -1.81$	0.07

ASI-6 = Addiction Severity Index; CSA+ = crack cocaine dependence with a history of childhood sexual abuse; CSA– = crack cocaine dependence without a history of childhood sexual abuse; SD = standard deviation.
*Wilcoxon Mann Whitney test unless otherwise indicated.
†$n = 77$.

In general we found reduced GDNF and NGF plasma levels and a sustained increase in BDNF levels during early abstinence in women with crack dependence compared with controls. The ANCOVAs, adjusted for age and BMI, showed that at 4 days of detoxification, both the CSA+ and CSA– groups presented lower levels of GDNF than the control group ($F_{2, 107}$ = 3.79; p = 0.26). At the eleventh day, however, GDNF increased in the CSA+ group, while GDNF levels in the CSA– group remained reduced compared with controls ($F_{2, 111}$ = 9.74, p < 0.001). The GDNF levels continued to increase for the CSA+ group after 18 days of detoxification ($F_{2, 105}$ = 12.20, p < 0.001; Fig. 1A). Since there were differences regarding relapse history between these 2 groups, we compared the fractional GDNF change [(time2 – time1 ÷ time2 × time1) × 100] in the second and third week of follow-up regardless of whether they had prior hospital admissions owing to relapse, and we did not find any significant association (data not shown). In addition, both the CSA+ and CSA– groups exhibited a sustained increase in BDNF levels compared with controls since they showed higher plasmatic concentrations at 4 days ($F_{2, 109}$ = 8.15, p = 0.001), 11 days ($F_{2, 113}$ = 5.36, p = 0.006) and 18 days ($F_{2, 112}$ = 5.49, p = 0.005) of detoxification (Fig. 1B). Conversely, we identified lower levels of NGF in both the CSA+ and CSA– groups compared with the control group at 4 days ($F_{2, 108}$ = 13.0, p < 0.001), 11 days ($F_{2, 109}$ = 12.5, p < 0.001) and 18 days ($F_{2, 101}$ = 11.95, p < 0.001; Fig. 1C). Moreover, there were no differences among the groups in levels of NT3 (all p > 0.05; Fig. 1D), although NT4/5 levels were found to be higher in the CSA+ group than the CSA– group at the end of detoxification (Fig. 1E).

Discussion

This study investigated several NFs during early abstinence in women with crack cocaine dependence with and without a history of childhood sexual abuse and in a group of healthy controls. Overall GDNF, NGF, NT-3 and NT4/5 levels were lower in women with crack cocaine dependence than controls at all time points, whereas levels of BDNF were higher. However, we found that GDNF plasma levels in women with crack cocaine dependence who reported a history of

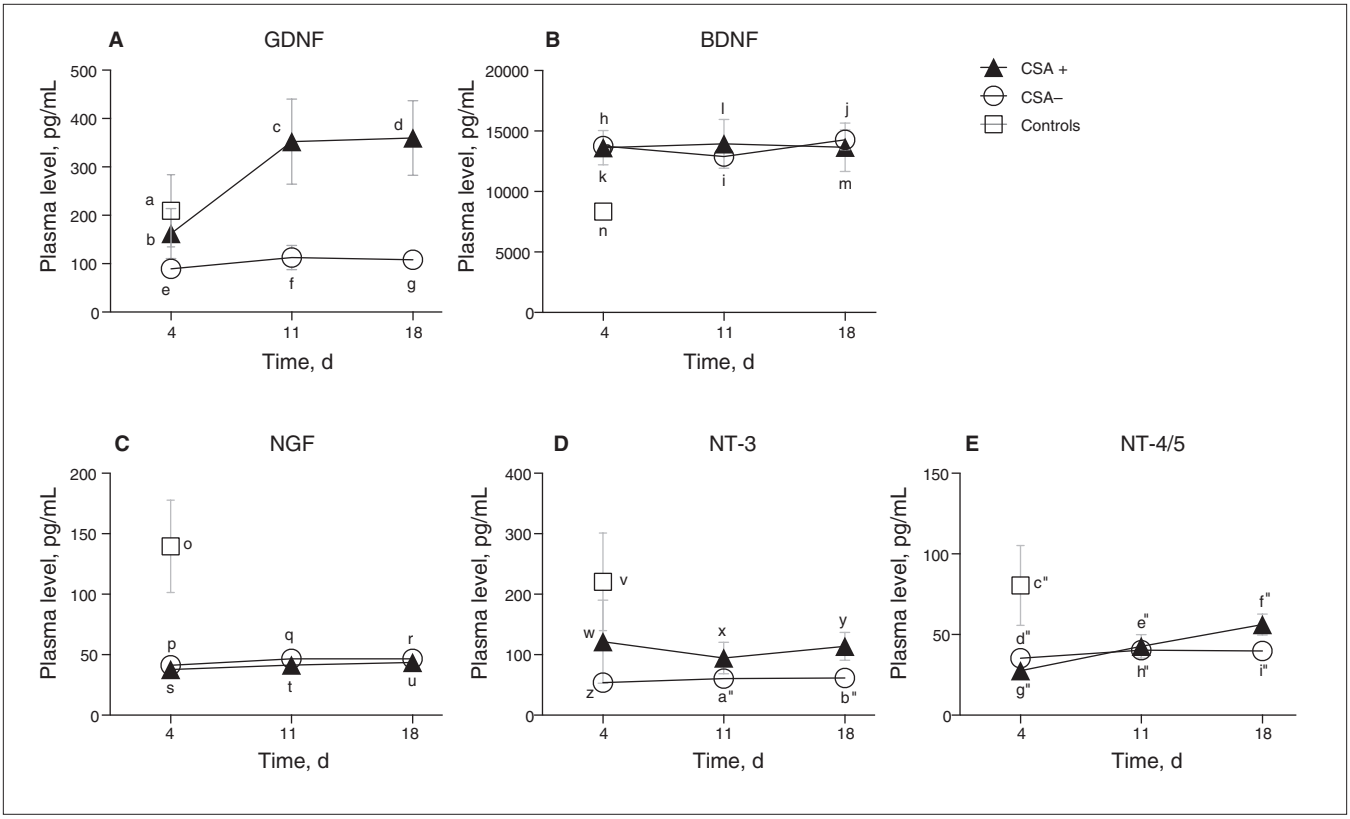

Fig. 1: General linear models, adjusted for age and body mass index, showing differences in neurotrophic factors plasma levels between women with crack cocaine dependence with (CSA+) and without (CSA–) a history of childhood sexual abuse and healthy controls**.** All data are presented raw (means and standard errors of the mean). Repeated-measures analyses of covariance (ANCOVAs) showed independent pairwise comparisons among the estimated marginal means. **(A)** Effect of group: CSA+ > CSA–, p = 0.001; effect of time: [18 d], [11 d] > [4 d], all p < 0.001. **(B–D)** No specific effect of group or time. **(E)** No specific effect of group; effect of time: day 18 and day 11 greater than day 4, all p < 0.005. These analyses revealed a significant group × time interaction effect for glial cell line–derived neurotrophic factor (GDNF; p = 0.001) and neurotrophic factor-4/5 (NT-4/5; p = 0.004) levels and a significant general effect of time for nerve growth factor (NGF; p = 0.026). The examination of group differences at separate time points using ANCOVAs with revealed significant differences among the 3 groups: **(A)** c greater than f (p < 0.001); d greater than g and a (all p < 0.05); **(B)** h–m greater than n (all p < 0.005); **(C)** o greater than p–u (all p < 0.005); **(D)** no pairwise comparison effects; and **(E)** f" greater than i" (p = 0.012). NT-3 = neurotrophic factor-3.

childhood sexual abuse increased dramatically during 3 weeks of detoxification, whereas those without a history of childhood sexual abuse showed lower and stable levels of GDNF under the same conditions of detoxification.

Glial cell line–derived neurotrophic factor is the most prominent member of the transforming growth factor-β1 superfamily. It is a potent survival and differentiation factor for dopaminergic neurons during development and is the first — and so far the only — NF that can rescue dopaminergic neurons from degeneration in adults.[35] Long-term exposure to cocaine diminishes the dopaminergic components of the striatum and midbrain.[36] There is also evidence suggesting that smoking crack cocaine carries a higher risk of neurotoxicity than cocaine use alone.[37] Therefore, an increase in GDNF could be associated with a compensatory mechanism operating during detoxification. Such an increase, however, was detected only in the CSA+ group. Relevant for this specific finding, animal models of early life stress have been associated with reduced DA transporter binding in the ventral striatum, elevated baseline DA levels and increased DA release in response to acute stress, which were all correlated with enhanced sensitivity to cocaine in adulthood.[38] In addition, when the neuroprotective effects of exercise were investigated in a rat model of Parkinson disease, rats exposed to early life stress showed a reduced adaptive response associated with neuroprotection in the striatum, a major target of DA neurons.[39] Furthermore, maternal separation has been related to 50% reduction of GDNF concentration in the cerebellum of mice,[40] and it has been shown to alter the dopaminergic system by reducing surface expression of the DA active transporter and its affinity, increasing the amount of time required to clear DA from the extracellular fluid in the striatum.[41] Interestingly, when abstaining patients with cocaine dependence and a history of childhood adversity were compared with those who did not report early adverse family experiences, those with early life adversity exhibited reduced levels of the DA metabolite homovanillic acid and higher levels of prolactin, cortisol and adrenocorticotropic hormone.[42,43] Altogether, these data support the idea that early life stress could be related to DA dysfunction in adulthood, which could help explain our GDNF results. Supporting this hypothesis, data from monkeys exposed to methamphetamine for 2 days at either a young age, an adult age or at midgestation demonstrated that at midgestation and in adulthood, DA neurons were susceptible to methamphetamine-induced damage. However, DA neurons in young animals appeared totally resistant to the treatment, and the authors suggested that elevated levels of GDNF, which were detected only in young animals, could explain this neuroprotective effect.[44]

Interestingly, when GDNF was overexpressed in the nucleus accumbens of mice, only chronic stressed animals exhibited an increase in social interaction and sucrose preference,[45] reflecting an association between chronic stress, GDNF expression and reward system. In addition, activation of the GDNF pathway results in synaptic remodelling and alterations in the responsiveness of the mesolimbic dopaminergic system, which leads to an attenuation of biochemical and behavioural changes observed upon exposure of psychostimulants.[46] Despite the absence of a clear explanation of why GDNF plasma level in the CSA+ group was higher than than in the CSA– group and of why both groups had lower levels than controls, we hypothesized that GDNF levels could reflect changes in DA-related signalling associated with early life stress. In addition, we found that the severity of childhood sexual abuse predicted higher levels of GDNF after detoxification independently of age, BMI, severity of cocaine addiction and use of medication.

Our finding of sustained elevation of BDNF plasma levels in women with cocaine dependence is consistent with those of many previous clinical and preclinical reports.[4,6,17,19] A consistent body of evidence stemming from rat models has indicated that elevated BDNF plays an important role in the enhancement of mesolimbic DA neuronal function, which could be related both to increased drug seeking behaviour/craving and to the reward effect of the drug.[47] The role of DA is primarily in the reinforcing effects of drugs and the motivation to obtain them. Thus, a potential effect of BDNF on DA neurons may be the neuronal plasticity and synaptic modifications induced by BDNF expression in the DA reward circuit, an essential link in the drive–reward paradox of addiction. Furthermore, it has already been suggested that elevated serum BDNF levels in individuals recovering from cocaine dependence are a predictive marker of future cocaine relapse outcomes.[4] In agreement with this idea, infusion of exogenous BDNF in the nucleus accumbens and ventral tegmental area (VTA) was associated with the reinstatement of cocaine seeking behaviour in rodents.[18] Recent reports have investigated the molecular mechanisms underlying such cocaine-induced alterations of BDNF transcription in the VTA.[48,49] For example, BDNF messenger RNA (mRNA) expression in the striatum and VTA is associated with cocaine-induced alterations in chromatin remodelling, including histone acetylation.[19,48] Acetylation of histone proteins produces an open chromatin conformation and increased gene transcription.[50] Hence, it is possible that the cocaine-induced neuronal plasticity is mediated in part by changes in the chromatin structure surrounding mesolimbic BDNF promoters, which could lead to increased BDNF transcription.[17]

Although BDNF expression may reflect a compensatory mechanism in response to cocaine-related neurotoxicity, the effects of BDNF on cocaine withdrawal could be brain region–specific. Thus the augmented BDNF expression might regulate both the positive and negative effects of neuronal activity and synaptic plasticity in a site-specific manner.[47] For instance, in a rat model of depression, BDNF has an antidepressant effect when infused into the hippocampus and a prodepressant effect when infused into the VTA.[51] In the same way, BDNF infusion into the dorsomedial prefrontal cortex suppresses cocaine seeking despite pro–drug seeking effects of subcortical BDNF infusion.[47] Those results indicate that BDNF also regulates neuroadaptations that are critical in mediating or preventing cocaine-induced dysfunctional neuroadaptations in the reward system.

Recent evidence suggests that cocaine addiction may involve progressive drug-induced neuroplasticity of the striatum and nucleus accumbens.[52,53] It has been well described that infusion of BDNF and NT4/5 in the VTA decreases cyclic adenosime monophosphate–dependent protein kinase

and adenylyl cyclase activity in the nucleus accumbens, both of which are increased by chronic cocaine exposure.[54] In addition, BDNF and NT4/5 intrastriatal treatment can modify neurotransmitter-related gene expression when dopaminergic neurons are damaged in the rat striatum.[55] Therefore hypothetically the increase in BDNF and the lower level of other NFs observed in this study could be related to self-regulation mechanisms associated with cocaine withdrawal.

In addition, it has been shown that neurotrophins bind to 2 different classes of receptor proteins: tropomyosin receptor kinase (Trk) receptors and the p75 neurotrophin receptor.[56] This dual system allows the transduction of different signals; cell death is signalled through p75, whereas cell survival is signalled through Trk receptors. Three Trk genes have been identified in mammals (*TrkA*, *TrkB* and *Trk*C). Nerve growth factor stimulates TrkA receptors, BDNF and NT4/5 stimulate TrkB receptors, and NT-3 activates TrkC and, less potently, TrkA and TrkB.[57] However, the activation of the same Trk receptor by different ligands seems to trigger different signalling events, because different adaptor molecules can be specifically recruited for TrkA, TrkB, or TrkC.[58] For example, activation of TrkB by BDNF or NT-4/5 does not always lead to identical biological effects.[58] In fact, occupation of TrkB by BDNF or NT-4/5 modulates the permeability of an ion channel associated with TrkB. Interestingly, it has been suggested that BDNF and NT-4/5 could be involved in some form of competition.[59] Therefore, the discrepancy found between the levels of BDNF and the other neurotrophins in our results could reflect different signalling cascades activated through Trk receptors.

Most of the literature on BDNF has investigated its role in many psychiatric disorders, especially mood disorders, but data regarding other NF changes are insufficient and sometimes contradictory. A clinical study reported elevated NGF levels in older healthy participants exposed to chronic stress,[60] whereas other studies on major depression reported lower serum levels of NGF.[61–63] On the other hand, peripheral GDNF was found to be increased in patients with late-onset depression compared with controls,[64] whereas other studies found GDNF to be decreased in individuals with major depression.[65] In addition, reduced expression of GDNF and NT-3 mRNA of peripheral blood cells in patients with major depression were associated with depressive state,[66] whereas increased cerebrospinal fluid levels of NT-3 were found in elderly patients with major depression.[67] Patterns of increased versus decreased levels of peripheral NFs should be considered carefully since such changes may reflect dynamic physiologic mechanisms.

It is also important to consider that altered NF expression could be a response to chronic stress. For patients with crack dependence, a 3-week period of crack cocaine withdrawal could be characterized as a chronic stress state. Therefore, the sustained elevation of BDNF plasma levels encompasses an increase in HPA axis tone in response to stress, including an altered immunoendocrine response, suggesting that BDNF is also an important biomarker of HPA axis functioning.[4] In addition, repeated stress might result in a long-term downregulation of NGF levels and upregulation of NT-3.[68,69]

There is evidence that peripheral NF concentrations are associated with brain tissue expression.[70] Indeed, cerebral BDNF crosses the blood–brain barrier, and it has been suggested that peripheral BDNF levels are correlated with brain tissue BDNF levels in rodents.[70] Moreover NGF, NT3, and NT-4/5 can cross the blood–brain barrier of mice in vivo to arrive at the brain parenchyma, and peripheral administration of neurotrophins could have neurobiological effects within the central nervous system.[71] However, plasma BDNF has been hypothesized to behave as a state-dependent marker, while serum BDNF might signify illness.[72] Such differences in serum and plasma BDNF expression could explain the findings of similar studies that assessed serum BDNF levels but did not find elevated BDNF expression during the period of early cocaine detoxification in humans.[3,8]

Limitations

It is important to consider the limitations of our study. First, because we used convenience sampling in a women's psychiatric unit to recruit participants, our sample included only women; therefore, we cannot conclude that our findings are applicable to men. Second, it is noteworthy that more than half of the patients with crack cocaine dependence had a comorbid psychiatric diagnosis. However, we did not find any significant effect of comorbid psychiatric disorders on NF levels. Third, some of the women with crack dependence were using neuroleptics, mood stabilizers or antidepressants, and use of these medications can impact the levels of some NFs,[62,73] which could partially explain the NF changes that we found. It should be noted that the 2 groups of women with crack dependence were homogeneously treated with common medications, and we did not find any significant differences between the experimental groups regarding the use of these medications. Furthermore, it is extremely difficult to investigate nonmedicated inpatients during the acute phase of drug withdrawal. However, 42 patients were medication-free (12 in the CSA+ group and 30 in the CSA– group), therefore ANCOVAs including childhood sexual abuse as a between-subjects factor and covarying for age and BMI were used to analyze single parameters in each point of assessment for all NFs (data not shown). We found similar results regarding GDNF measures; we also found that NT-4/5 was higher in the CSA+ group than the CSA– group at the end of detoxification. In contrast, NT-3 levels were higher in the CSA+ group at all time points; however, this result should be interpreted with caution owing to the small size of the CSA+ sample. In sum, these effects for almost all NFs assessed seem not to be driven entirely by medication use, although the role of NT-3 in this association remains unclear. Fourth, blood samples were obtained from controls only once. Given that NF levels can be affected by many environmental factors, repeated measures would have been ideal to show that levels in controls remained constant throughout the period of study. Fifth, we did not investigate the possible confounding effects of other forms of stress during childhood, such as parental loss or other types of maltreatment. Finally, the possibility of memory bias could have influenced the retrospective rating of childhood sexual abuse exposure in the CTQ assessment. It is important to note, however, that this study

was conducted with a careful methodological design that controlled for several clinical factors that could bias the results.

Conclusion

Despite these limitations, our study documented that during 3 weeks of early recovery from crack cocaine abuse, GDNF plasma levels increased significantly in the women with crack dependence and a history of childhood sexual abuse who behaviourally reported more impairment from crack consumption, but GDNF levels were reduced in those without a history of childhood sexual abuse. The BDNF plasma levels also remained elevated in both groups of women with crack dependence, whereas NGF remained reduced in these groups compared with the control group. Nonsignificant marginal reduction in NT-3 and NT-4/5 plasma levels could be observed in women with crack dependence. Although the BDNF results replicated previous findings, it is worth pointing out that our data showed that BDNF levels were stable and much higher in patients with crack cocaine dependence during early abstinence than at baseline. To our knowledge, however, the GDNF data are novel in humans with crack cocaine dependence. Although increased BDNF levels have previously been reported as a marker of cocaine relapse risk,[4,19] no clinical study has reported an association between elevated GDNF expression and drug relapse. This is important since data from rodents indicated that during the first weeks of withdrawal from cocaine self-administration, GDNF-dependent neuroadaptations in midbrain dopaminergic neurons played an important role in the development of incubation of cocaine craving and future relapse.[74] Further studies with larger samples and that include relapse follow-up measures are needed to support such findings, especially regarding the role of early life stress on GDNF expression and the possibility that the GDNF and other NF pathways (e.g., astrocytic basic fibroblast growth factor) may be a new and promising target for the treatment of drug addiction.

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