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Enhanced nicotine-seeking behavior following pre-exposure to repeated cocaine is accompanied by changes in BDNF in the nucleus accumbens of rats ☆

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ABSTRACT

We investigated the behavioral and molecular interactions between cocaine and nicotine, through evaluating locomotor activity, nicotine intravenous self-administration and gene expression. Locomotor sensitization was induced in male Wistar rats by repeated cocaine (20 mg/kg; i.p.) or saline injections once a day over 7 days. Three days after the last injection, rats were challenged with either saline or cocaine (15 mg/kg; i.p.) and the locomotor activity was measured. The very next day animals received either saline or nicotine (0.4 mg/kg; s.c.) and the locomotor cross-sensitization was tested. Animals were then prepared with intrajugular catheters for nicotine self-administration. Nicotine self-administration patterns were evaluated using fixed or progressive ratio schedules of reinforcement and a 24-h unlimited access binge. Immediately after the binge sessions animals were decapitated, the brains were removed and the nucleus accumbens was dissected. The dynorphin (DYN), μ-opioid receptor (mu opioid), neuropeptide Y (NPY), brain-derived neurotrophic factor (BDNF), tropomyosin-related tyrosine kinase B receptor (TrkB) and corticotropin-releasing factor receptor type 1 (CRF-R1) gene expression were measured by the reverse transcription-polymerase chain reaction (RT-PCR). Pretreatment with cocaine caused sensitization of cocaine motor response and locomotor cross-sensitization with nicotine. In the self-administration experiments repeated cocaine administration caused an increase in the nicotine break point and nicotine intake during a 24 h binge session.

1. Introduction

An important issue in the field of drug abuse research is the characterization of risk factors related to increased vulnerability to drug addiction (Anthony and Petronis, 1995). There is growing clinical evidence suggesting that previous exposure to cocaine increase the vulnerability to nicotine addiction. Controlled human studies have demonstrated that acute cocaine administration increases cigarette smoking (Roll et al., 1996). Moreover, cocaine-dependent smokers often report smoking more cigarettes during cocaine use (Budney et al., 1993; Higgins et al., 1994; Torchalla et al., 2011).

Pre-clinical studies also provide evidences for cocaine-induced increase in the vulnerability to nicotine abuse and addiction. For example, in rhesus monkeys, higher rates of combined nicotine and cocaine self-administration were observed, relative to isolated cocaine or nicotine self-administration (Freeman and Woolverton, 2009; Mello and Newman, 2011).

In rats, several studies have demonstrated the effects of nicotine exposure on cocaine self-administration (Horger et al., 1992; Anker and Carroll, 2011). For example, it has been reported that nicotine exposure increases the acquisition rates of cocaine self-administration (Horger et al., 1992) and its break point under a progressive-ratio (PR) schedule of reinforcement (Anker and Carroll, 2011). While, several studies have demonstrated the influence of nicotine exposure on cocaine self-administration, the effect of previous exposure to cocaine on nicotine self-administration has been poorly investigated.

Repeated nicotine administration may also lead to a sensitized locomotor response following psychostimulant challenge (Santos et al., 2009). This phenomenon is termed behavioral cross-sensitization. Behavioral sensitization is suggested to reflect neuroadaptive processes

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associated to drug addiction (Robinson and Berridge, 1993). Recently, it was demonstrated that nicotine priming enhances cocaine-induced behavioral sensitization. However the effect of previous cocaine treatment on nicotine-induced locomotor sensitization has not demonstrated yet.

Many studies indicate that neuroadaptations of the mesocorticolimbic dopamine system are related to drug abuse and addiction (Wise, 2009; Pierce and Kumaresan, 2006; Nestler, 2001; Kalivas, 2007). In this way, it has been demonstrated that repeated cocaine or nicotine exposure produces many long-lasting alterations in the mesocorticolimbic system that contribute to addiction-like behaviors (Cao et al., 2011; Koya et al., 2009; Guez-Barber et al., 2011).

It has been proposed that cocaine and nicotine produce persistent alterations in the mesolimbic system due to changes in gene expression (Russo et al, 2010; Dreyer, 2010). Studies suggest that administration simultaneous of equipotent doses of nicotine and cocaine, produce additive effects on nucleus accumbens dopamine release (Sziraki et al., 1999; Gerasimov et al., 2000). However, change in gene expression, associated with the effect of cocaine on increased vulnerability to nicotine addiction is still poorly understood.

Cocaine and nicotine modulate the release of neurotransmitters, which activate their different receptors, leading to the activation of transcription factors. Transcriptional activation in the mesocorticolimbic system has been associated to neural plasticity related to the development of drug addiction (Chandrasekar and Dreyer, 2009).

Indeed, repeated cocaine or nicotine administration may change the expression of neurotransmitters (e.g. dynorphine (DYN), neuropeptide Y (NPY)), receptors (e.g. μ-opioid receptor, tropomyosin-related tyrosine kinase B receptor (TrkB) and corticotropin-releasing factor receptor type 1 (CRF-R1)), and transcription factors (e.g. brain-derived neurotrophic factor (BDNF)) (Ang et al., 2001; Ghitza et al., 2010; Hope et al., 1994; Houdi et al., 1998; Kivinummi et al., 2011; Nestler, 2005a, 2005b, 2008; Shippenberg and Rea, 1997). These changes may increase drug-seeking behavior.

Thus, the goal of the present study was to investigate the behavioral and molecular changes that result from the interactions between cocaine and nicotine. To this end we evaluated whether the pretreatment with cocaine could modify cocaine- or nicotine-induced locomotor activity, nicotine intravenous self-administration, and the expression of DYN, mu opioid, NPY, BDNF, TrkB and CRF-R1 genes.

2. Materials and methods

2.1. Subjects

Male Wistar rats, 225–250 g at arrival, obtained from the animal breeding facility of the Univ. Estadual Paulista — UNESP were individually housed in plastic cages 19 cm (width) × 30 cm (length) × 14 cm (height).

Rats were continuously maintained on a reversed light cycle (12-h:12-h, lights off at 08:00 a.m.) with controlled temperature (21 °C) and humidity (35–40%), with unrestricted access to food and water. During the experiment, rats received 18 g rat chow per day provided in their home cage after each daily experimental session. This feeding schedule results in the gradual weight gain of approximately 15 g/week (Donny et al., 1995). Unlimited access to water was available throughout all experiments. All experiments were performed during the dark phase.

The experimental protocol was approved by the Ethics Committee for Use of Human or Animal Subjects of the School of Pharmaceutical Science — UNESP (CEP-19/2008).

2.2. Locomotor response to cocaine and nicotine

2.2.1. Apparatus

Locomotor activity measures were conducted in commercially available (Columbus Instruments, Columbus, OH, USA) activity monitoring chambers, consisting of Plexiglas cages. The chambers, measuring 44 cm (width) × 44 cm (length) × 20 cm (height) cm included 10 pairs of photocells beams, which were used to measure the horizontal locomotor activity. The consecutive interruption of two beams was recorded as one locomotor count.

2.2.2. Locomotor measurement

Rats were pretreated with cocaine (20 mg/kg; i.p.) or saline for 7 days (Marin et al., 2008). Three days after the last cocaine (COC) or saline (SAL) administration, rats were challenged with saline (COC–SAL n = 10; SAL–SAL n = 10) or cocaine (15 mg/kg; i.p.) (COC–COC n = 10; SAL–COC n = 10). Immediately following the injections, animals were put in an activity chamber and their locomotor activity was recorded during a 30-minute testing session as described above. In the very next day the same animals received saline (COC–SAL n = 10; SAL–SAL n = 10) or nicotine (NIC) (0.4 mg/kg; s.c.) (COC–NIC n = 10; SAL–NIC n = 10). Immediately following the injections, animals were put in an activity chamber and their locomotor activity was recorded during a 15-minute testing session as described above. In both tests, animals were allowed a 20-minute habituation period to the photocell apparatus immediately prior to injections. Animals from different groups were tested randomly during the dark phase between 10:00 a.m. and 14:00 p.m.

2.3. Intravenous drug self-administration

Seven days after the locomotor test, animals were subjected to intravenous nicotine self-administration procedures. The general procedure was adapted from George et al. (2007).

2.3.1. Apparatus

For nicotine self-administration the animals were put individually in Plexiglas experimental chambers (30 × 30.5 × 24.5 cm), enclosed in light- and sound attenuating boxes. The floor of the chambers consisted of a Plexiglas tray covered with sawdust. A hole in the ceiling allowed the passage and free movement of the tethered catheter (Strategic Applications Inc., Libertyville, IL, USA) that was connected to a counterbalanced swivel and an infusion pump (Insight Equipments®, Ribeirão Preto, SP, Brazil). The front wall of the chamber contained one interchangeable panel. The panel was equipped with two levers, located 5 cm from the floor, two cue lights (red and green) above each lever and a session light in the middle of the panel (12 cm from the floor).

2.3.2. Drug

(−) — Nicotine 99% was obtained from Sigma-Aldrich (St Louis, MO, USA). The dose of nicotine was chosen based on previous experiments conducted in our laboratory (Leão et al., 2012).

2.3.3. Training

Training consisted of three 60-minute training sessions, in which each response on the active lever (alternated between left and right sides) was reinforced with the delivery of 0.2 ml sucrose (6%) (fixed ratio schedule of reinforcement; FR 1), followed by a 10-second time-out. Each rat was allowed continuous access to sucrose solution during the entire 60-minute. Responding on the inactive lever had no scheduled consequence.

2.3.4. Surgery

Twenty-four hours after the last training session, cocaine and saline pretreated rats were implanted with permanently indwelling catheters (Silastic™ silicon tubing, inner diameter = 0.63 mm, outer diameter = 1.17 mm) into the right jugular vein under a combination of ketamine (100.0 mg/kg) and xylazine (6.0 mg/kg) anesthesia. The catheter was passed subcutaneously to the rat's back where it exited through a small incision and was affixed to a plastic pedestal

(Strategic Applications Inc., Libertyville, IL, USA) mounted inside a harness system (Strategic Applications Inc., Libertyville, IL, USA). Rats were allowed to recover from surgery for 7 days in their home cage with free access to food and water. To prevent inflammation and infection rats received cetoprophen 1% (5.0 mg/kg; i.m.), and cefazolin (10.0 mg/kg; i.v.) for three consecutive days following surgery.

The catheter was flushed daily with heparinized saline (20 IU/ml) and 0.2 ml of saline in order to maintain the catheter's patency.

2.3.5. Nicotine self-administration

After recovering from surgery all rats were initially given unlimited access to sucrose (6%) self-administration, in which each lever press was reinforced with the delivery of 0.2 ml sucrose (6%) (fixed ratio schedule of reinforcement; FR 1), followed by a 10-second time-out. During self-administration sessions, a green cue light above the active lever signaled sucrose availability and a red cue light, also above the active lever, signaled sucrose delivery. During the time-out period, the green and red cue lights were extinguished and lever press were recorded but had no consequences. Each daily session was terminated after 60-minute of access. After three sessions of sucrose reinforcement nicotine self-administration was assessed during 3-hour sessions. Initially, nicotine (0.03 mg/kg per infusion) was infused (0.1 ml/s) associated to sucrose 6% (0.2 ml, v.o.) delivered on schedule of reinforcement (FR1) for the active lever. Responses on the inactive lever were recorded but had no programmed consequence. Each daily session was terminated after 10 drug infusions or 3-hour of access, whichever occurred first. Simultaneous administration of sucrose and nicotine sessions was ended after completing 10 drug infusions within 3-hour over two consecutive days. Following this period only nicotine (0.03 mg/kg per infusion) was infused (0.1 ml/s) on schedule of reinforcement (FR1) for the active lever. After completing 10 infusions of nicotine within 3-hour over two consecutive days, the FR schedule was progressively increased to fixed ratio 3 (FR 3). Rats were maintained for at least four additional days on a limited access on FR 3 schedule before being examined during a progressive ratio schedule of reinforcement. The acquisition and maintenance took about 15 days.

2.3.6. Progressive ratio schedule of drug reinforcement

After the acquisition and maintenance phase, self-administration according to a progressive ratio (PR) schedule of drug reinforcement was verified. The progression of response requirements followed the algorithm: 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26… The final infusion delivered was defined as the break point. The PR session was terminated once the rat failed to obtain an infusion during 60-minute. The average number of total responses and the last ratio completed across three PR trials for each individual rat was calculated.

2.3.7. Twenty-four hour unlimited access drug "binge"

After the final PR session, each rat was allowed one additional day of limited drug access (0.03 mg/kg per infusion of nicotine, FR3 schedule, total of 10 infusions). The very next day, a 24-hour binge protocol was implemented starting approximately at 10:00 a.m. Each rat was allowed continuous access to drug infusions (0.03 mg/kg per infusion — 0.1 ml/s) on FR1 schedule during the entire 24-hour binge. The amounts of drug self-administered as well as the pattern of responding were recorded.

2.4. Real time-polymerase chain reaction (RT-PCR)

Posterior, RT-PCR was used to determine changes of DYN, mu opioid, NPY, BDNF TrKB and CRF-R1, twenty-four hour after the "binge" session. Briefly, animals were killed by rapid decapitation and the nucleus accumbens was isolated, frozen in liquid nitrogen and stored in -80C freezer for posterior RT-PCR assay. mRNA was extracted homogenizing individual samples in TRIzol® Reagent (Invitrogen, cat# 15596-026) according to the manufacturer's instructions. Briefly, TRIzol® Reagent was added to each sample and the aqueous layer was isolated following centrifugation. Total mRNA was precipitated with isopropanol in the presence of linear acrylamide overnight. Samples were centrifuged and the extracted mRNA pellets were washed with 70% ethanol and re-suspended in diethylpyrocarbonate water. Total mRNA was quantified and its quality was examined through the ratio 260/280 and the integrated of the bands was seen in gel of agarose. Next the total mRNA was reverse-transcribed into cDNA with random hexamers using Superscript III (Invitrogen, Carlsbad, CA, USA). The primer sequences used to amplify DYN, mu opioid, NPY, BDNF TrKB and CRF-R1, were listed in Table 1. Real-time RT-PCR was performed in a total volume of 25 μl buffer solution containing 5 μl of template cDNA, 12.5 μl 2× SYBR Green Master mix (Applied Biosystems, Foster City, CA, USA), 1.5 μl MQ and 15 ρM of each primer. The absolute cDNA quantities were determined, using standard curves, with Applied Bioscience 7500 System Software. Cycle thresholds (Ct) were calculated from triplicate reactions using the second derivative of the amplification curve. DYN, mu opioid, NPY, BDNF TrKB and CRF-R1 Ct values were normalized to GAPDH Ct values (DCt) since GAPDH was not regulated by cocaine/nicotine. Fold changes were calculated using the DDCt method as described (Applied Biosystems, Carlsbad, CA, USA).

2.5. Statistics

Data from the locomotor activity were analyzed by two-way ANOVA for repeated measure considering pretreatment (saline vs. cocaine) and time after saline and cocaine or nicotine test injections as the repeated factor. When a significant ($p<0.05$) main effect was observed F-tests for contrast analysis were applied.

Nicotine self-administration and gene expression data were analyzed using Student's *t*-tests comparing saline and cocaine groups. Significant differences are reported for $p<0.05$.

3. Results

3.1. Locomotor activity

3.1.1. Cocaine locomotor sensitization

Two-way ANOVA revealed significant differences for pretreatment [$F(1,15)=4.91; p<0.05$] and time after injection [$F(1,15)=17.33; p<0.001$] factors. In addition, the interaction between factors was also detected [$F(1,15)=7.03; p<0.001$].

Further analysis (F-Test) showed that rats pretreated with cocaine showed significantly higher cocaine–induced locomotor activity when compared to the saline pretreated ones from 5 to 20 min after cocaine injection (Fig. 1), evidencing that the pretreatment with cocaine caused sensitization to its motor response ($p<0.05$).

Table 1
The primer sequences used to amplify DYN, mu opioid, NPY, BDNF, TrkB and CRF-R1 genes.

Genes	5′-3′ sequence
CRFR1-For	TTGGCAAACGTCCTGGGGTAT
CRFR1-Rev	GCGGACAATGTTGAAGAGAAAG
BDNF-For	TCACAGCGGCAGATAAAAAGACT
BDNF-Rev	GTGTCTATCCTTATGAACCGCCAGCCAA
TrKb-For	CCTCGTTGGAGAAGATCAAG
TrKb-Rev	CGTGGTACTCCGTGTGATTG
Dyn-For	CTCTCCAGCAGGTTTGGC
Dyn-Rev	CTGGGACCGAGTCACCAC
Miopio-For	TTACGGCCTGATGATCTTACGA
Miopio-Rev	GGTGATCCTGCGCAGATTC
NPY-For	GGGGCTGTGTGGACTGACCCT
NPY-Rev	GATGTAGTGTCGCAGAGCGGAG
GAPDH-For	ATGGGAAGCTGGTCATCAAC
GAPDH-Rev	ACGCCAGTAGACTCCACGAC

3.1.2. Nicotine locomotor cross-sensitization

Two-way ANOVA did not reveal significant differences for the pretreatment factor [$F(1,15) = 2.90$; $p<0.05$]. However, ANOVA revealed a main effect of time after injection [$F(1,15) = 5.56$; $p<0.05$]. ANOVA did show the interaction between factors [$F(1,15) = 2.13$; $p<0.05$].

Further analysis (F-Test) showed that rats pretreated with cocaine showed significantly higher nicotine–induced locomotor activity when compared to the saline pretreated ones in the first 5 min (Fig. 2), evidencing the cross-sensitization between cocaine and nicotine ($p<0.05$).

3.2. Nicotine self-administration

3.2.1. Progressive ratio schedule

Fig. 3 depicts the number of responses and the last ratio achieved (break point) in a progressive ratio schedule to nicotine in animals pretreated with cocaine or saline.

Student *t*-test revealed a significant increase in the number of responses to obtain nicotine in the cocaine pretreated group when compared to the saline pretreated one ($t(15) = 5.06$; $p<0.01$). In addition, the last ratio achieved was higher in the cocaine group as compared to the saline one ($t(15) = 5.32$; $p<0.001$) (Fig. 3).

3.2.2. Twenty-four hour unlimited access nicotine ("binge")

Fig. 4 depicts the number of responses, reinforcement and the total nicotine intake during 24-hour "binge" session in cocaine or saline pretreated animals.

Student *t*-test revealed a significant increase in the number of responses ($t(15) = 2.19$; $p<0.05$), reinforcements ($t(15) = 2.78$; $p<0.05$), and nicotine intake ($t(15) = 2.80$; $p<0.05$), in the cocaine pretreated rats when compared to the saline pretreated ones.

3.3. Real time-polymerase chain reaction

Fig. 5 depicts the quantitative RT-PCR of DYN, mu opioid, NPY, BDNF, TrKB and CRF-R1 twenty-four hours after the "binge" session.

Student t-tests revealed a significant increase in BDNF expression in the cocaine pretreated rats when compared to the saline pretreated ones ($t(11) = 2.61$; $p<0.05$). The expression of DYN, mu opioid, NPY, TrkB or CRF-R1 was not significantly modified by the pre-treatment with cocaine.

4. Discussion

In the present study we evaluated whether the pretreatment with cocaine could modify cocaine- or nicotine– induced locomotor activity, nicotine intravenous self-administration, and the expression of DYN, mu opioid, NPY, BDNF, TrkB and CRF-R1 genes (Fig. 6).

Repeated cocaine administration resulted in cocaine and nicotine locomotor sensitization followed by an enhanced in nicotine break-point and intake during a 24 h binge session. Furthermore, these alterations in nicotine addiction-like behaviors were accompanied by a selective increase in BDNF expression in the nucleus accumbens. Our results suggest that previous cocaine exposure may increase the vulnerability to nicotine addiction.

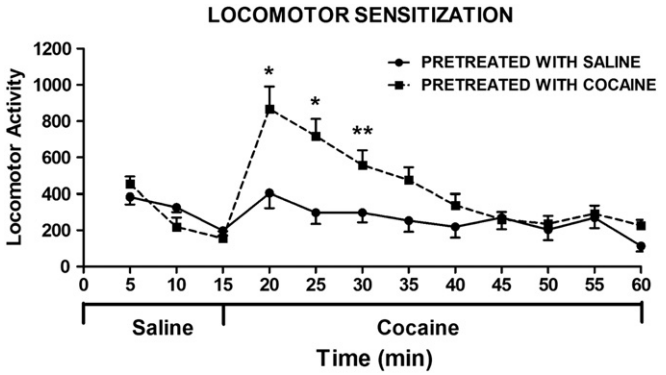

Fig. 2. Locomotor activity following saline or cocaine (15 mg/kg; i.p.) challenge injections in rats pretreated with repeated cocaine (20 mg/kg; i.p.; daily during 7 days) or saline administration. Rats were habituated in the activity-monitoring chamber for 20 min before the saline challenge injection. Data represent mean ± SEM (N = 10–11 animals per group) of locomotion counts accumulated in 5-minute intervals immediately after injections. * $p<0.01$ compared to rats pretreated with saline; ** $p<0.05$ compared to rats pretreated with saline.

In rodents, depend on the dose, repeated exposure to drugs of abuse may induce behavioral sensitization, that has been defined as a progressive and enduring enhancement of the motor stimulant effects of these drugs (Robinson and Berridge, 2000).

Our results are in accordance with other showing that repeated cocaine injections cause locomotor sensitization (Brown et al., 2011; Marin et al., 2008, 2009; Wuo-Silva et al., 2011). However, an important finding of our study is the observation of cross-sensitization between cocaine and nicotine. There are evidences that repeated nicotine injections increase the locomotor effects of cocaine. For instance, it has been shown that adult and adolescent rats pre-treated with nicotine displayed locomotor sensitization to psychostimulants, such as, cocaine or amphetamine (Collins and Izenwasser, 2004; Santos et al., 2009). However, Levine et al. (2011) showed in mice, that the pretreatment with cocaine did not sensitize the locomotor response to nicotine. The lack of consensus between our results and Levine's results may be due to differences in the species used (rats vs. mice), and nicotine challenge dose and route. To our knowledge, we have shown for the first time that the repeated pretreatment with cocaine causes cross-sensitization to nicotine.

Considering that behavioral sensitization is thought to reflect molecular and cellular changes involved with the increase in the motivation to drug self-administration (Robinson and Berridge, 2000, 2001, 2008; Moussawi et al., 2009), we investigate whether repeated cocaine exposure could also increase the motivation and self-administration.

We observed that previous repeated cocaine administration combined with the nicotine self-administration caused an increase in nicotine break point. Although nicotine self-administration in rodents has been demonstrated in a number of laboratories (see Le Foll and Goldberg, 2009 for review), to our knowledge this is the first study to demonstrate that repeated cocaine exposure increases nicotine self-administration.

In our study, the PR schedule revealed a significant increase in the break-point in rats pretreated with cocaine relative to those pretreated with saline, suggesting that they may be more motivated to obtain

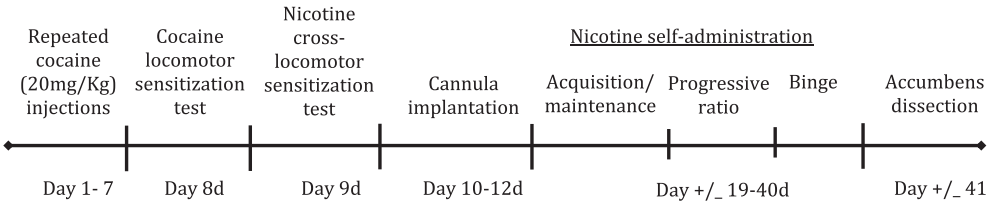

Fig. 1. Experimental protocol.

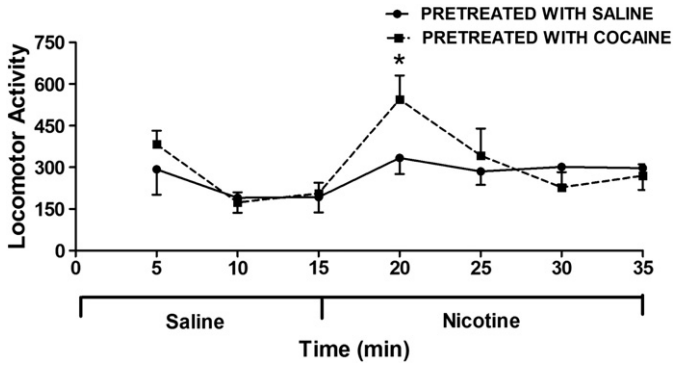

Fig. 3. Locomotor activity following saline or nicotine (0.4 mg/kg; s.c.) challenge injections in rats pretreated with repeated cocaine (20 mg/kg) or saline administration. Rats were habituated in the activity-monitoring chamber for 20 min before the saline challenge injection. Data represent mean ± SEM (N = 10–11 animals per group) of locomotion counts accumulated in 5-minute intervals immediately after injections. * $p<0.01$ compared to rats pretreated with saline.

nicotine when pre-exposed to cocaine. In the same way, it has been demonstrated that previous exposure to nicotine increased the PR response to cocaine self-administration (Bechtholt and Mark, 2002). Although tobacco is considered highly addictive in humans, in animal models the reinforcing efficacy of nicotine is weak (Robinson and Pritchard, 1992). Thus our data, showing that cocaine-induced increase in nicotine the PR schedule is quite relevant since they corroborate the clinical hypothesis that cocaine intake is a risk factor for tobacco addiction (Cousins et al., 2001).

Similarly, studies carried out in rhesus monkeys, showed that combined self-administration of nicotine and cocaine was higher relative to isolated cocaine or nicotine (Freeman and Woolverton, 2009; Mello and Newman, 2011). This interaction has also been demonstrated to other drugs, as previously reported by Henningfield and Griffiths (1981). These authors showed that the previous exposure to methylphenidate increased nicotine-taking in a fixed ratio schedule.

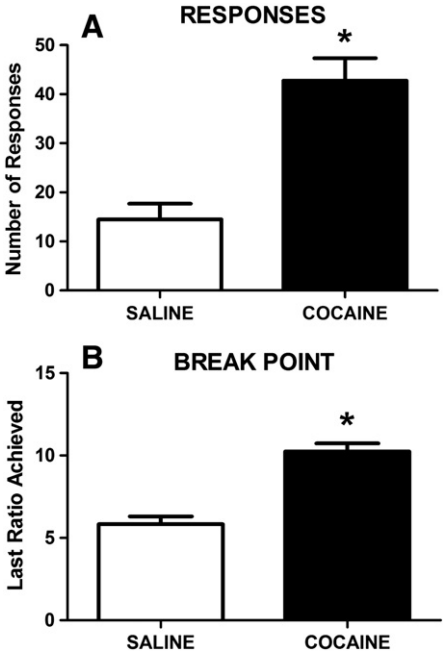

Fig. 4. Number of responses (A) and the last ratio achieved (B) in a progressive ratio schedule to nicotine in animals pretreated with repeated cocaine (20 mg/kg; i.p.; daily during 7 days) or saline administration. Data represent mean ± SEM (N = 7–8 animals per group). * $p<0.05$ compared to saline group.

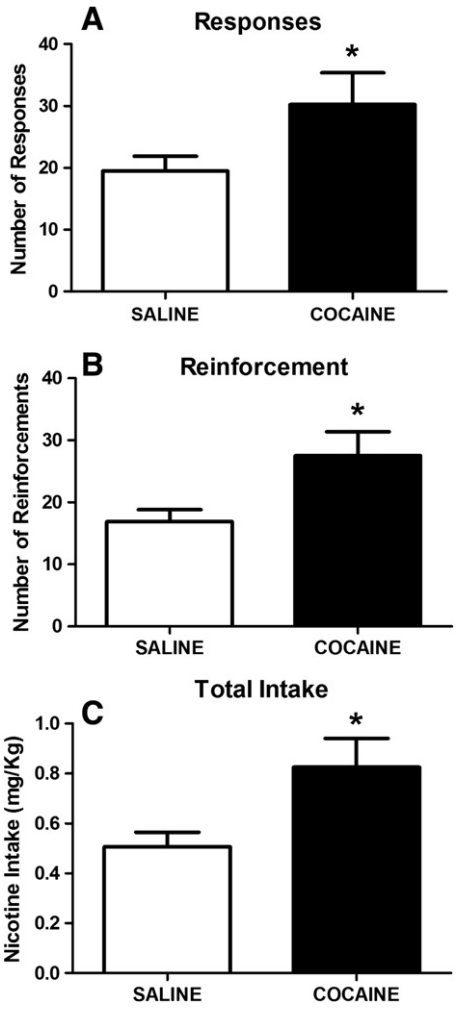

Fig. 5. Number of responses (A), reinforcement (B) and the total nicotine intake (C) during 24-hours "binge" session in animals pretreated with repeated cocaine (20 mg/kg; i.p.; daily during 7 days) or saline administration. Data represent mean ± SEM (N = 7–8 animals per group). * $p<0.05$ compared to saline group.

We observed that repeated cocaine treatment also caused escalation of nicotine-taking behavior during a 24 h binge session, which was accompanied by persistent increase in lever pressing response. The 24-h/day-access model is relevant, in special for nicotine self-administration, because it is able to evaluate possible interferences of the circadian pattern, which appears to be present in human smokers (Chandra et al., 2007; Frederiksen and Frazier, 1977; Morgan et al., 1985).

This increase in nicotine intake could be due to cocaine withdrawal symptoms, which could do the rats take more nicotine to relief these symptoms. But more studies should be performed to address this issue.

Also, evidence has shown that previous nicotine administration can increase the cocaine reward, measured by conditioned place preference (Levine et al., 2011). Taken together, these findings suggest that the repeated exposure to these substances can promote similar neuroadaptations in the brain reward pathway. Since, studies have demonstrated that the capacity of drugs and other environmental manipulations to promote increased sensitivity to drug's stimulant effects and escalation of drug intake has been associated with neuroadaptations in the dopaminergic mesolimbic system (Nestler, 2005a,b; Thomas et al., 2008). Repeated cocaine or nicotine exposure can promote alterations on selected signaling cascades and growth factors of excitatory transmission implicated in neuroplasticity underlying addiction-related behaviors (Bhang et al., 2010; Malenka and Bear, 2004; Muñoz et al., 2011; Poo, 2001; Sweatt, 2009).

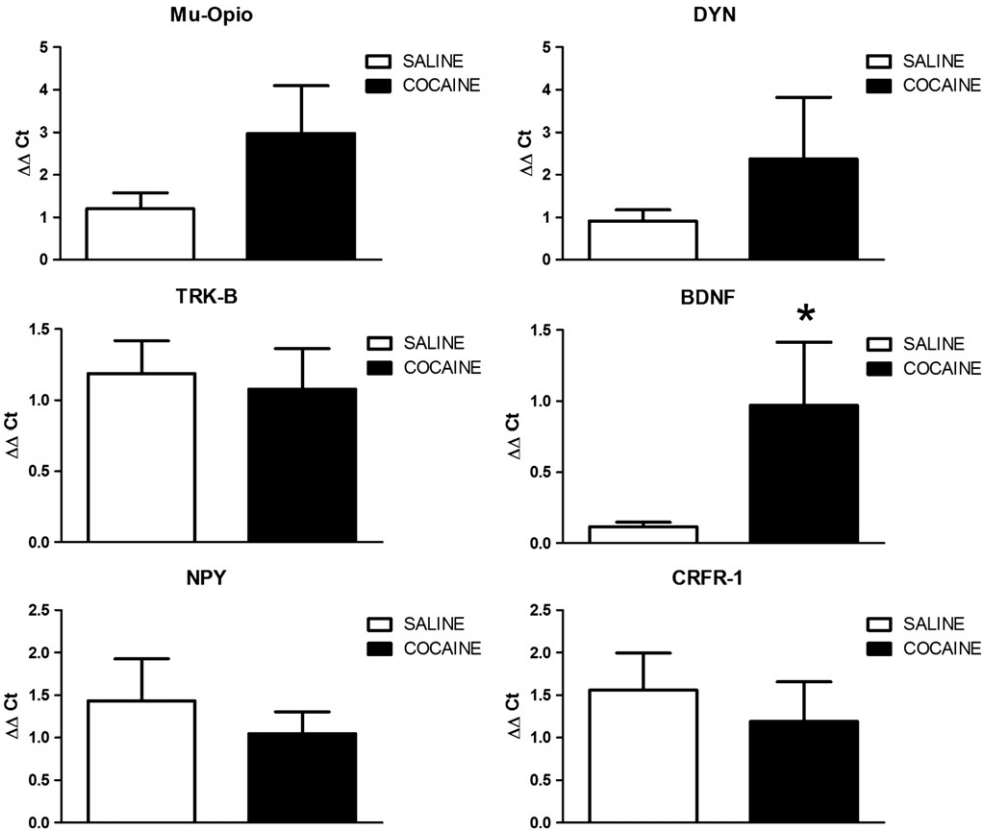

Fig. 6. Quantitative RT-PCR of DYN, mu Opioid, NPY, BDNF, TrkB and CRF-R1 24 hour after the "binge" session, of the nucleus accumbens of rats pretreated with saline or cocaine. Data represent mean ± SEM (N = 6 animals per group). * $p<0.05$ when compared to saline group.

Multiple lines of evidence suggest that BDNF is involved in processes of addiction, craving and withdrawal (Grimm et al., 2003; Horger et al., 1999; Pu et al., 2006). Evidences show an association between nicotine dependence and alterations in BDNF expression level (Andresen et al., 2009; Bhang et al., 2010; Kenny et al., 2000).

Our results show a selective increase in the levels of BDNF mRNA twenty-four hours after the end of the "binge" session in rats pretreated with cocaine. These results suggest that pretreatment with cocaine on its own or in combination of nicotine self-administration increased BDNF mRNA levels in the nucleus accumbens. Indeed, it has been demonstrated that contingent and noncontingent cocaine exposure increase BDNF protein levels in the striatum (Graham et al., 2007; Liu et al., 2006; Zhang et al., 2002). Moreover, it was demonstrated that repeated exposure to cocaine produced long-lasting increase in BDNF levels in the nucleus accumbens that is associated with enhanced responsiveness to cocaine self-administration (Grimm et al., 2003). In addition, Richardson and Roberts (1996) studies showed that injections of BDNF into the nucleus accumbens increased rats' motivation to work for cocaine in a progressive ratio reinforcement schedule of cocaine self-administration. Recently, a clinical study reported increased BDNF plasma levels in abstinent smokers (Bhang et al., 2010). In animals, it was demonstrated that repeated daily injections nicotine increased accumbal BDNF in adolescent mice (Correll et al., 2009). Furthermore, Kivinummi et al. (2011) showed that a chronic oral nicotine treatment, which promotes increase in nicotine intake, promoted enhanced BDNF levels in the nucleus accumbens.

Unfortunately, our experimental design makes difficult to state which manipulation could be mediating these alterations. Future studies in our laboratory will specifically address this issue.

Although, studies have showed that BDNF-induced neural plasticity is mediated by the TrkB receptor and that repeated cocaine administration increase TrKB expression (Crooks et al., 2010), we did not find up-regulation of this gene.

Although we have not found changes in DYN, mu opioid, NPY, TrKB and CRF-R1 mRNA levels, other studies have demonstrated their involvement in the behavioral effects of nicotine. For example, blockade of the brain stress system using a corticotropin-releasing factor-1 (CRF1) receptor antagonist decreases nicotine intake in rats (George et al., 2007). Similarly, the antagonism of opioid neurotransmission by pretreatment with naloxonazine, a mu opioid antagonist, significantly reduced the number of nicotine infusions during a self-administration session (Liu and Jernigan, 2011). Moreover, it has been demonstrated that nicotine locomotor sensitization was accompanied by downregulation in NPY mRNA levels in the medial nucleus of amygdala (Aydin et al., 2011).

Overall, pretreatment with cocaine induced sensitized locomotor response to cocaine and nicotine, and escalated of nicotine self-administration accompanied by enhancement of BDNF expression in of accumbens neurons.

5. Conclusion

Pretreatment with cocaine caused sensitization of cocaine motor response and locomotor cross-sensitization with nicotine. In the self-administration experiments repeated cocaine administration caused an increase in the nicotine break point and nicotine intake during a 24 h binge session.

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References

Andresen JH, Loberg EM, Wright M, Goverud IL, Stray-Pedersen B, Saugstad OD. Nicotine affects the expression of brain-derived neurotrophic factor mRNA and protein in the hippocampus of hypoxic newborn piglets. J Perinat Med 2009;37:553–60.

Ang E, Chen J, Zagouras P, Magna H, Holland J, Schaeffer E, et al. Induction of nuclear factor-kappaB in nucleus accumbens by chronic cocaine administration. J Neurochem 2001;79:221–4.

Anker JJ, Carroll ME. Adolescent nicotine exposure sensitizes cue-induced reinstatement of cocaine seeking in rats bred for high and low saccharin intake. Drug Alcohol Depend 2011;118:68–72.

Anthony JC, Petronis KR. Early-onset drug use and risk of later drug problems. Drug Alcohol Depend 1995;40:9-15.

Aydin C, Oztan O, Isgor C. Vulnerability to nicotine abstinence-related social anxiety-like behavior: molecular correlates in neuropeptide Y, Y2 receptor and corticotropin releasing factor. Neurosci Lett 2011;490:220–5.

Bechtholt AJ, Mark GP. Enhancement of cocaine-seeking behavior by repeated nicotine exposure in rats. Psychopharmacology (Berl) 2002;162:178–85.

Bhang SY, Choi SW, Ahn JH. Changes in plasma brain-derived neurotrophic factor levels in smokers after smoking cessation. Neurosci Lett 2010;468:7-11.

Brown TE, Lee BR, Mu P, Ferguson D, Dietz D, Ohnishi YN, et al. A silent synapse-based mechanism for cocaine-induced locomotor sensitization. J Neurosci 2011;31:8163–74.

Budney AJ, Higgins ST, Hughes JR, Bickel WK. Nicotine and caffeine use in cocaine-dependent individuals. J Subst Abuse Treat 1993;5:117–30.

Cao J, Dwyer JB, Mangold JE, Wang J, Wei J, Leslie FM, et al. Modulation of cell adhesion systems by prenatal nicotine exposure in limbic brain regions of adolescent female rats. Int J Neuropsychopharmacol 2011;14:157–74.

Chandra S, Shiffman S, Scharf DM, Dang Q, Shadel WG. Daily smoking patterns, their determinants, and implications for quitting. Exp Clin Psychopharmacol 2007;15: 67–80.

Chandrasekar V, Dreyer JL. microRNAs miR-124, let-7 d and miR-181a regulate cocaine-induced plasticity. Mol Cell Neurosci 2009;42:350–62.

Collins SL, Izenwasser S. Chronic nicotine differentially alters cocaine-induced locomotor activity in adolescent vs. adult male and female rats. Neuropharmacology 2004;46: 349–62.

Correll JA, Noel DM, Sheppard AB, Thompson KN, Li Y, Yin D, et al. Nicotine sensitization and analysis of brain-derived neurotrophic factor in adolescent beta-arrestin-2 knockout mice. Synapse 2009;63:510–9.

Cousins MS, Stamat HM, de Wit H. Acute doses of d-amphetamine and bupropion increase cigarette smoking. Psychopharmacology 2001;157:243–53.

Crooks KR, Kleven DT, Rodriguiz RM, Wetsel WC, McNamara JO. TrkB signaling is required for behavioral sensitization and conditioned place preference induced by a single injection of cocaine. Neuropharmacology 2010;58:1067–77.

Donny EC, Caggiula AR, Knopf S, Brown C. Nicotine self-administration in rats. Psychopharmacology (Berl) 1995;122:390–4.

Dreyer JL. New insights into the roles of microRNAs in drug addiction and neuroplasticity. Genome Med 2010;2:92.

Frederiksen LW, Frazier M. Temporal distribution of smoking. Addict Behav 1977;2: 187–94.

Freeman KB, Woolverton WL. Self-administration of cocaine and nicotine mixtures by rhesus monkeys. Psychopharmacology (Berl) 2009;207:99-106.

George O, Ghozland S, Azar MR, Cottone P, Zorrilla EP, Parsons LH, et al. CRF-CRF1 system activation mediates withdrawal-induced increases in nicotine self-administration in nicotine-dependent rats. Proc Natl Acad Sci USA 2007;104:17198–203.

Gerasimov MR, Franceschi M, Volkow ND, Rice O, Schiffer WK, Dewey SL. Synergistic interactions between nicotine and cocaine or methylphenidate depend on the dose of dopamine transporter inhibitor. Synapse 2000;38:432–7.

Ghitza UE, Zhai H, Wu P, Airavaara M, Shaham Y, Lu L. Role of BDNF and GDNF in drug reward and relapse: a review. Neurosci Biobehav Rev 2010;35:157–71.

Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. Nat Neurosci 2007;10:1029–37.

Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y. Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci 2003;23:742–7.

Guez-Barber D, Fanous S, Golden SA, Schrama R, Koya E, Stern AL, et al. FACS identifies unique cocaine-induced gene regulation in selectively activated adult striatal neurons. J Neurosci 2011;31:4251–9.

Henningfield JE, Griffiths RR. Cigarette smoking and subjective response: effects of d-amphetamine. Clin Pharmacol Ther 1981;30:497–505.

Higgins ST, Budney AJ, Hughes JR, Bickel WK, Lynn M, Mortensen A. Influence of cocaine use on cigarette smoking. JAMA 1994;272:1724.

Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, et al. Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. Neuron 1994;13:1235–44.

Horger BA, Giles MK, Schenk S. Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology (Berl) 1992;107: 271–6.

Horger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, Taylor JR. Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. J Neurosci 1999;19:4110–22.

Houdi AA, Dasgupta R, Kindy MS. Effect of nicotine use and withdrawal on brain preproenkephalin A mRNA. Brain Res 1998;799:257–63.

Kalivas PW. Cocaine and amphetamine-like psychostimulants: neurocircuitry and glutamate neuroplasticity. Dialogues Clin Neurosci 2007;9:389–97. [Review].

Kenny PJ, File SE, Rattray M. Acute nicotine decreases, and chronic nicotine increases the expression of brain-derived neurotrophic factor mRNA in rat hippocampus. Brain Res Mol Brain Res 2000;85:234–8.

Kivinummi T, Kaste K, Rantamäki T, Castrén E, Ahtee L. Alterations in BDNF and phospho-CREB levels following chronic oral nicotine treatment and its withdrawal in dopaminergic brain areas of mice. Neurosci Lett 2011;491:108–12.

Koya E, Uejima JL, Wihbey KA, Bossert JM, Hope BT, Shaham Y. Role of ventral medial prefrontal cortex in incubation of cocaine craving. Neuropharmacology 2009;1: 177–85.

Le Foll B, Goldberg SR. Effects of nicotine in experimental animals and humans: an update on addictive properties. Handb Exp Pharmacol 2009;192:335–67. [Review].

Leão RM, Cruz FC, Marin MT, Planeta Cda S. Stress induces behavioral sensitization, increases nicotine-seeking behavior and leads to a decrease of CREB in the nucleus accumbens. Pharmacol Biochem Behav 2012;101:434–42.

Levine A, Huang Y, Drisaldi B, Griffin Jr EA, Pollak DD, Xu S, et al. Molecular mechanism for a gateway drug: epigenetic changes initiated by nicotine prime gene expression by cocaine. Sci Transl Med 2011;3:107–9.

Liu X, Jernigan C. Activation of the opioid μ1, but not δ or κ, receptors is required for nicotine reinforcement in a rat model of drug self-administration. Prog Neuropsychopharmacol Biol Psychiatry 2011;35:146–53.

Liu QR, Lu L, Zhu XG, Gong JP, Shaham Y, Uhl GR. Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. Brain Res 2006;1067:1-12.

Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron 2004;44:5-21. [Review].

Marin MT, Cruz FC, Planeta CS. Cocaine-induced behavioral sensitization in adolescent rats endures until adulthood: lack of association with GluR1 and NR1 glutamate receptor subunits and tyrosine hydroxylase. Pharmacol Biochem Behav 2008;91: 109–14.

Marin MT, Berkow A, Golden SA, Koya E, Planeta CS, Hope BT. Context-specific modulation of cocaine-induced locomotor sensitization and ERK and CREB phosphorylation in the rat nucleus accumbens. Eur J Neurosci 2009;30:1931–40.

Mello NK, Newman JL. Discriminative and reinforcing stimulus effects of nicotine, cocaine, and cocaine + nicotine combinations in rhesus monkeys. Exp Clin Psychopharmacol 2011;19:203–14.

Morgan SF, Gust SW, Pickens RW, Champagne SE, Hughes JR. Temporal patterns of smoking topography in the natural environment. Int J Addict 1985;20:613–21.

Moussawi K, Pacchioni A, Moran M, Olive MF, Gass JT, Lavin A, et al. N-Acetylcysteine reverses cocaine-induced metaplasticity. Nat Neurosci 2009;12:182–9.

Muñoz MÁ, Sanjuan R, Fernández-Santaella MC, Vila J, Montoya P. Neuropsychological aspects of nicotine craving. Adicciones 2011;23:111–23.

Nestler EJ. Molecular neurobiology of addiction. Am J Addict 2001;10:201–17. [Review].

Nestler EJ. Is there a common molecular pathway for addiction? Nat Neurosci 2005a;8: 1445–9.

Nestler EJ. The neurobiology of cocaine addiction. Sci Pract Perspect 2005b;3:4-10. [Review].

Nestler EJ. Transcriptional mechanisms of addiction: role of DeltaFosB. Philos Trans R Soc Lond B Biol Sci 2008;363:3245–55. [Review].

Pierce RC, Kumaresan V. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 2006;30:215–38.

Poo MM. Neurotrophins as synaptic modulators. Nat Rev Neurosci 2001;2:24–32. [Review].

Pu L, Liu QS, Poo MM. BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal. Nat Neurosci 2006;9:605–7.

Richardson NR, Roberts DC. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods 1996;66:1-11. [Review].

Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 1993;18:247–91. [Review].

Robinson TE, Berridge KC. The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 2000;2:S91-S117. [Review].

Robinson TE, Berridge KC. Incentive-sensitization and addiction. Addiction 2001;96: 103–14. [Review].

Robinson TE, Berridge KC. The incentive sensitization theory of addiction: some current issues. Philos Trans R Soc Lond B Biol Sci 2008;363:3137–46. [Review].

Robinson JH, Pritchard WS. The role of nicotine in tobacco use. Psychopharmacology (Berl) 1992;108:397–407. [Review].

Roll JM, Higgins ST, Budney AJ, Bickel WK, Badger GJ. A comparison of cocaine-dependent cigarette smokers and non-smokers on demographic, drug use and other characteristics. Drug Alcohol Depend 1996;40:195–201.

Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. Trends Neurosci 2010;33:267–76.

Santos GC, Marin MT, Cruz FC, Delucia R, Planeta CS. Amphetamine- and nicotine-induced cross-sensitization in adolescent rats persists until adulthood. Addict Biol 2009;14: 270–5.

Shippenberg TS, Rea W. Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. Pharmacol Biochem Behav 1997;57: 449–55. [Review].

Sweatt JD. Experience-dependent epigenetic modifications in the central nervous system. Biol Psychiatry 2009;65:191–7.

Sziraki I, Sershen H, Benuck M, Hashim A, Laitha A. Differences in receptor system participation between nicotine- and cocaine-induced dopamine overflow in nucleus accumbens. NY Acad Sci 1999;877:800–2.

Thomas MJ, Kalivas PW, Shaham Y. Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. Br J Pharmacol 2008;154:327–42. [Review].

Torchalla I, Strehlau V, Okoli CT, Li K, Schuetz C, Krausz M. Smoking and predictors of nicotine dependence in a homeless population. Nicotine Tob Res 2011;13: 934–42.
Wise RA. Roles for nigrostriatal-not just mesocorticolimbic-dopamine in reward and addiction. Trends Neurosci 2009;32:517–24.
Wuo-Silva R, Fukushiro DF, Borçoi AR, Fernandes HA, Procópio-Souza R, Hollais AW, et al. Addictive potential of modafinil and cross-sensitization with cocaine: a pre-clinical study. Addict Biol 2011;16:565–79.
Zhang D, Zhang L, Lou DW, Nakabeppu Y, Zhang J, Xu M. The dopamine D1 receptor is a critical mediator for cocaine-induced gene expression. J Neurochem 2002;82:1453–64.