Neutralization of Neutrophin-3 in the Ventral Tegmental Area or Nucleus Accumbens Differentially Modulates Cocaine-Induced Behavioral Plasticity in Rats

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ABSTRACT These experiments were designed to assess the influence of neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) in the mesoaccumbens dopamine system on the initiation of behavioral sensitization to cocaine. A neutralizing antibody for NT-3, BDNF or their vehicle was administered into the ventral tegmental area (VTA) or nucleus accumbens prior to each of four daily injections of 15 mg/kg cocaine. Behavioral sensitization was operationally defined as a significant increase in the behavioral response to cocaine relative to the first daily injection. Results indicated that the NT-3 antibody had differential effects when administered into the VTA or nucleus accumbens. Intra-VTA microinjection of anti-NT-3 resulted in enhanced sensitization to repeated cocaine injections in that the cocaine-induced behavioral response in the anti-NT-3 group was significantly greater than the vehicle group following the second and third daily injections of cocaine. Administration of anti-NT-3 into the nucleus accumbens increased the behavioral response to cocaine over all 4 days of cocaine administration, with no sensitization of this behavioral response. In contrast, pretreatment with anti-BDNF into the VTA or nucleus accumbens had no influence on the initiation of behavioral sensitization to cocaine. Taken together, these data indicate that neutralization of NT-3 in the VTA enhances cocaine-induced behavioral sensitization, while administration of the NT-3 antibody into the nucleus accumbens increases the hyperactive behavioral response induced by cocaine but impairs the further development of behavioral sensitization. **Synapse 46:57–65, 2002.**

INTRODUCTION

Behavioral sensitization refers to the augmentation of behavioral hyperactivity following repeated injections of psychostimulants such as cocaine or amphetamine. Psychostimulant sensitization alters the reinforcing effects of these drugs (Mendrek et al., 1998; Lorrain et al., 2000), which suggests that the neurobiological changes underlying behavioral sensitization may overlap with those underlying cocaine craving and addiction (Robinson and Berridge, 1993). Brain dopamine systems are involved in both the reinforcing and locomotor-stimulating effects of psychostimulants (Wise and Bozarth, 1987; Koob, 1988) and several converging lines of evidence indicate that the mesoaccumbens dopamine system is critically involved in the development (initiation) and maintenance (expression) of psychostimulant-induced behavioral sensitization (Kalivas and Stewart, 1991; White et al., 1995; Pierce and Kalivas, 1997). Initiation of behavioral sensitization refers to the transient sequence of cellular and molecular events precipitated by psychostimulant administration that ultimately leads to enduring changes in neuronal function. It is thought that the neurophysiological processes that contribute to the initiation of

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behavioral sensitization, although transient, are necessary to trigger other permanent changes in the mesotelencephalic dopamine systems that are directly involved in the long-term maintenance, or expression, of behavioral sensitization (Kalivas and Stewart, 1991; White et al., 1995). A variety of data show that initiation and expression of behavioral sensitization are both temporally and anatomically distinct. Initiation of behavioral sensitization to psychostimulants occurs in the ventral tegmental area (VTA) (Kalivas and Stewart, 1991; White et al., 1995), which is the locus of the dopaminergic cell bodies in the ventral midbrain that give rise to the mesocorticolimbic pathways (Fallon and Moore, 1978). In contrast, a growing number of neurochemical, electrophysiological, and molecular biological experiments indicate that several interconnected nuclei centered on the nucleus accumbens contribute to the expression of behavioral sensitization to psychostimulants (Nestler, 1997; Pierce and Kalivas, 1997; White and Kalivas, 1998).

Recent evidence indicates that neurotrophins play important roles in the initiation of behavioral sensitization to psychostimulants. For example, three once-daily microinjections of neutrotrophin-3 (NT-3) into the VTA, but not the substantia nigra, resulted in an enhanced behavioral response to a cocaine injection 2 weeks later (Pierce et al., 1999). These results suggest that the changes in the VTA in response to repeated NT-3 are similar to the changes induced by repeated exposure to cocaine. An acute injection of cocaine also resulted in a substantial increase in NT-3 mRNA levels in the VTA, implying that cocaine increases NT-3 synthesis (Pierce et al., 1999). The MAP kinase signal transduction cascade, which is activated by neurotrophins, including NT-3, is also involved in psychostimulant-induced behavioral sensitization. Intra-VTA administration of a MAP kinase kinase inhibitor prior to daily cocaine injections blocked the initiation of behavioral sensitization (Pierce et al., 1999). Consistent with this finding, repeated injections of cocaine, but not an acute injection, resulted in a significant increase in MAP kinase levels in the VTA (Berhow et al., 1996).

Brain-derived neurotrophic factor (BDNF) also influences behaviors modulated by the mesolimbic dopamine system. Continuous or repeated infusion of BDNF into the VTA or substantia nigra increased exploratory behavior (Martin-Iverson et al., 1994; Martin-Iverson and Altar, 1996; Horger et al., 1999; Pierce et al., 1999). Continuous infusion of BDNF into the VTA or substantia nigra also enhanced the behavioral hyperactivity induced by an acute injection of amphetamine (Altar et al., 1992; Martin-Iverson et al., 1994) or cocaine (Horger et al., 1999). However, when a withdrawal period was imposed following three daily microinjections of BDNF into the VTA, the behavioral response to a challenge injection of cocaine did not differ from control (Pierce et al., 1999). Collectively, these results indicate that although repeated or continuous administration of BDNF into the ventral midbrain augments the behavioral output of the mesotelencephalic dopamine system, it is not clear that BDNF-induced plasticity contributes to cocaine-induced behavioral sensitization.

The present experiments were designed to further evaluate the influence of NT-3 and BDNF in the mesolimbic dopamine system on cocaine-induced behavioral sensitization. Neutralizing antibodies raised against NT-3 or BDNF or their vehicle was administered into the VTA or nucleus accumbens during a sensitizing regimen of repeated daily cocaine injections.

MATERIALS AND METHODS

Animals and housing

Adult male Sprague-Dawley rats (250–300 g) were obtained from Taconic Farms (Germantown, NY) and housed individually in hanging stainless steel cages with food and water available ad libitum. A 12/12 light–dark cycle (lights on at 7:00 AM) was used; all of the experiments were performed during the light cycle. All experimental protocols were consistent with the guidelines issued by the U.S. National Institutes of Health and were approved by the Boston University School of Medicine Institutional Animal Care and Use Committee.

Surgery

Prior to surgery, the rats were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine and mounted in a stereotaxic apparatus. Stainless steel guide cannulae (14 mm, 24 gauge) were implanted bilaterally relative to bregma (Paxinos and Watson, 1997) 1 mm dorsal to the VTA (-6.0 A/P, ± 0.5 M/L, -7.0 D/V) or the medial nucleus accumbens ($+1.0$ A/P, ± 0.7 M/L, -5.5 D/V) and cemented in place by affixing dental acrylic to three stainless steel screws secured in the skull. Following surgery, the rats were allowed to recover for 4 days prior to the start of the sensitization regimen.

Behavioral testing/microinjections

The day prior to the start of the sensitization regimen the rats were habituated to the photocell boxes (AccuScan Instruments, Columbus, OH) for 3 h. On the first treatment day the rats were habituated to the photocell boxes for 1 h. Following habituation, the animals received a bilateral microinjection of anti-BDNF (500 ng/0.5 μl), anti-NT-3 (10 or 500 ng/0.5 μl) or vehicle (0.5 μl of 0.9% saline). For microinjection, the obturators were removed from the microinjection guide cannulae and replaced by injectors (33 gauge stainless steel), which extended 1 mm below the end of the guide cannulae into the VTA or medial nucleus accumbens. Bilateral infusions were made over 120 sec in a volume

of 0.5 μl/side. The injectors were left in place for 60 sec (to allow the compound to diffuse away from the tips of the cannulae) and then removed. Ten minutes after the microinjection the rats received an injection of cocaine (15 mg/kg, i.p.) and their behavior was monitored for 2 h postinjection.

Verification of cannulae placements

Following the completion of all microinjection experiments the animals were given an overdose of pentobarbital and perfused intracardially with 60 ml 0.9% saline followed by 60 ml 10% formalin. The brains were removed and coronal sections (100 μm) were taken at the level of the VTA or nucleus accumbens with a Vibratome (Technical Products International, St. Louis, MO). The sections were mounted on gelatin-coated slides and stained with Cresyl violet. An individual unaware of the animals' behavioral response determined cannula placements as well as potential drug- or cannula-induced neuronal damage.

Reagents

The antibodies used in these experiments included sheep anti-BDNF (Chemicon International, Temecula, CA, Cat. # AB1513P) and rabbit anti-NT-3 (Chemicon International, Cat. # AB1780SP). The BDNF and NT-3 antibodies were raised against recombinant human BDNF and NT-3. The antibodies were diluted in saline. The doses of these antibodies were chosen based on the manufacturer's suggested range of effective doses for inhibition of biological activity as well as previous in vitro and in vivo studies (Zhou et al., 1994, 1998; Zhou and Rush, 1995; Tafreshi et al., 1998). Cocaine was obtained as a gift from the National Institutes on Drug Abuse (Rockville, MD) and was dissolved in saline.

RESULTS

Intra-VTA administration of anti-NT-3 augments the initiation of behavioral sensitization to cocaine

Saline, anti-NT-3 (500 ng) or anti-BDNF (500 ng) was microinjected into the VTA 10 min prior to cocaine on Days 1 through 4. The behavioral responses recorded on Days 1 through 4 are summarized in Figure 1. The top panel depicts the total behavioral response recorded over the 120-min period following cocaine administration. These data were analyzed with a mixed factors analysis of variance (ANOVA). The between-subjects measure was drug treatment, the within-subjects measure was days of treatment. The results of this analysis revealed a significant main effect of drug treatment [$F(2,27) = 6.07$, $P < 0.0066$] and day [$F(3,81) = 18.9$, $P < 0.0001$] but no significant interaction between these factors [$F(6,81) = 1.09$, $P < 0.37$]. The significant main effect of day was assessed further by separate within-subjects ANOVAs across treatment days for the saline, anti-BDNF, and anti-NT-3 groups. The results of these analyses revealed significant main effects of day in the saline [$F(3,24) = 7.89$, $P < 0.0008$], anti-BDNF [$F(3,27) = 4.61$, $P < 0.0099$] and anti-NT-3 treatments [$F(3,30) = 8.54$, $P < 0.0003$]. Subsequent pairwise comparisons (Fisher's LSD) showed that the behavioral response on Day 4 was significantly greater than Day 1 in all three groups. In addition, the behavioral hyperactivity recorded on Day 3 in the anti-NT-3 group was significantly greater than Day 1. The significant main effect of treatment was assessed further by separate between-subjects ANOVAs across treatments for Days 1 through 4. The results of these analyses revealed significant main effects of treatment on Day 2 [$F(2,27) = 3.61$, $P < 0.041$] and Day 3 [$F(2,27) = 5.64$, $P < 0.009$], but not Day 1 [$F(2,27) = 0.41$, $P < 0.67$] or Day 4 [$F(2,27) = 2.49$, $P < 0.1$]. Subsequent pairwise comparisons (Fisher's LSD) of the Day 2 and Day 3 data revealed that the behavioral response recorded in the anti-NT-3 group was significantly greater than the saline group on that day. The middle and bottom panels summarize the time-courses of the behavioral responses on Days 1 and 3, respectively, of repeated cocaine administration in the saline/cocaine, anti-BDNF/cocaine, and anti-NT-3/cocaine groups. These data were analyzed with two-way mixed factors ANOVAs, with time as the repeated measure. The analysis of the data collected on Day 1 (Fig. 1, middle panel) showed a significant main effect of time [$F(11,297) = 4.94$, $P < 0.0001$] but no significant main effect of treatment [$F(2,27) = 0.41$, $P < 0.67$] and no significant interaction between these factors [$F(22,297) = 0.53$, $P < 0.96$]. The analysis of the Day 3 data (Fig. 1, bottom panel) revealed a significant main effect of treatment [$F(2,27) = 5.64$, $P < 0.009$] but no significant main effect of time [$F(11,297) = 1.23$, $P < 0.27$] and no significant interaction between these factors [$F(22,297) = 0.22$, $P < 0.99$]. There were 9–11 animals per group.

Intra-accumbal administration of anti-NT-3 increases cocaine-induced behavioral hyperactivity but impairs the initiation of behavioral sensitization to cocaine

Saline, anti-NT-3 (10 or 500 ng) or anti-BDNF (500 ng) was microinjected into the medial nucleus accumbens 10 min prior to cocaine on Days 1 through 4. The behavioral responses recorded on Days 1 through 4 are summarized in Figure 2. The top panel depicts the total behavioral response recorded over the 120-min period following cocaine administration. These data were analyzed with a mixed factors ANOVA. The between-subjects measure was drug treatment, the within-subjects measure was days of treatment. This analysis revealed a significant main effect of day [$F(3,99) = 14.75$, $P < 0.0001$] but no significant main effect of

treatment [F(3,33) = 1.59, $P < 0.21$] and no significant interaction between day and treatment [F(9,99) = 0.59, $P < 0.81$]. The significant main effect of day was analyzed further with separate within-subjects ANOVAs across treatment days. The results of these analyses revealed significant main effects of day in the saline/cocaine [F(3,30) = 4.11, $P < 0.015$], anti-BDNF/cocaine [F(3,24) = 8.37, $P < 0.0006$], and anti-NT-3-10/cocaine [F(3,18) = 5.77, $P < 0.006$] groups but not the anti-NT-3-500/cocaine group [F(3,27) = 1.34, $P < 0.28$]. Subsequent pairwise comparisons revealed significant differences between the behavioral responses on Days 1 and 4 in the saline/cocaine, anti-BDNF/cocaine, and anti-NT-3-10/cocaine groups (Fisher's LSD). The middle and bottom panels summarize the time-courses of the behavioral responses recorded on Days 1 and 4, respectively, in the saline/cocaine, anti-BDNF/cocaine and anti-NT-3-500/cocaine groups. These data were analyzed with two-way mixed factors ANOVAs, with repeated measures over time. The results of the analysis of the data collected on Day 1 (Fig. 2, middle panel) showed a significant main effect of drug treatment [F(2,27) = 3.33, $P < 0.05$], time [F(11,297) = 11.99, $P < 0.0001$] and a significant treatment × time interaction [F(22,297) = 2.92, $P < 0.0001$]. Subsequent pairwise comparisons (Fisher's LSD) demonstrated significant differences between the saline/cocaine and anti-NT-3-500/cocaine groups during the 10- and 20-min time points. The analysis of the Day 4 data (Fig. 2, bottom panel) revealed a significant main effect of time [F(11,297) = 26.31, $P < 0.0001$] and a significant day × time interaction [F(22,297) = 1.75, $P < 0.02$] but no significant main effect of drug treatment [F(2,27) = 0.19, $P < 0.82$]. Subsequent pairwise comparisons demonstrated significant differences between the saline/cocaine and anti-NT-3-500/cocaine groups during the 10- and 20-min time periods. There were 7–11 animals per group.

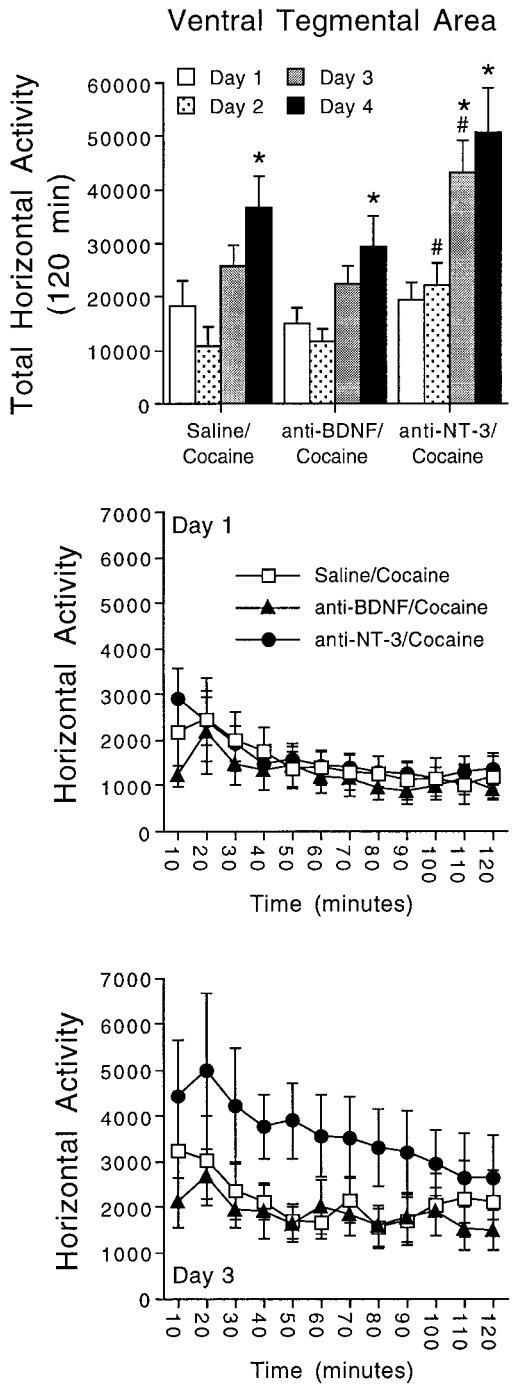


Fig. 1. Intra-VTA administration of anti-NT-3 augments the initiation of behavioral sensitization to cocaine. Saline, anti-BDNF (500 ng), or anti-NT-3 (500 ng) were microinjected into the VTA 10 min prior to cocaine (15 mg/kg, i.p.) on Days 1 through 4. The top panel depicts the total behavioral response recorded over the 120-min period following cocaine administration. Note the significant difference between the behavioral responses on Days 1 and 4 in the saline/cocaine, anti-BDNF/cocaine, and anti-NT-3/cocaine groups, the significant difference between Days 1 and 3 in the anti-NT-3/cocaine group, and the fact that the behavioral responses recorded on Days 2 and 3 in the anti-NT-3/cocaine group were significantly greater than the corresponding days in the saline/cocaine group. The middle and bottom panels summarize the time-courses of the behavioral responses on Days 1 and 3, respectively, of repeated cocaine administration in the saline/cocaine, anti-BDNF/cocaine, and anti-NT-3/cocaine groups. In the top panel the asterisks represent significant differences between Days 1 and 4 for that treatment group; the pound symbol represents a significant difference from the saline/cocaine group on that day (Fisher's LSD). There were 9–11 animals per group.

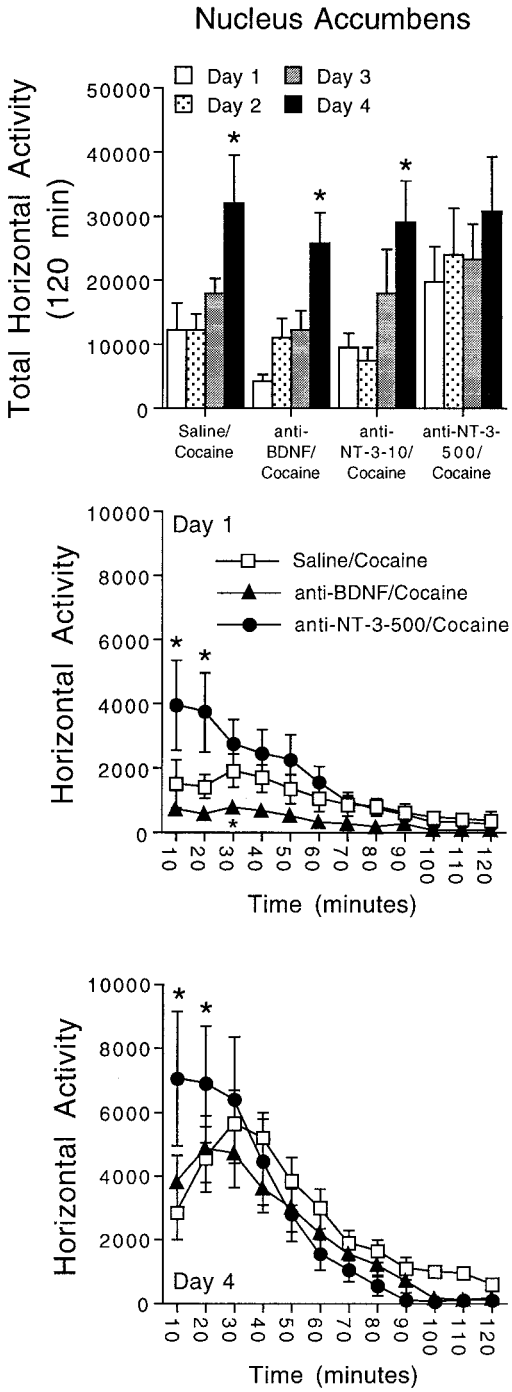
Intra-accumbal administration of anti-NT-3 does not influence spontaneous locomotor activity

Saline or anti-NT-3 (500 ng) was administered into the medial nucleus accumbens and behavioral activity was monitored for 2 h immediately following the microinjection. The total behavioral response (horizontal activity) recorded over the 2-h testing period was as follows (mean ± SEM): saline, 2377.5 ± 600.8, $n = 4$; anti-NT-3, 2077.5 ± 1027.3, $n = 4$. These data were analyzed with an unpaired t-test, which showed no

significant difference between these groups [$t(6) = 0.311$, $P < 0.77$].

Location of microinjection sites in the VTA and nucleus accumbens

The schematic brain sections depicted in Figure 3 are from the atlas of Paxinos and Watson (1997). The filled circles represent the placement of the tips of the microinjection cannulae located in the VTA (left) and nucleus accumbens (right). For the VTA, some of the cannulae tips were located at the border between the VTA and the medial substantia nigra, the ventral medial lemniscus, and the ventral red nucleus. For the nucleus accumbens, all of the cannulae tips were positioned in the medial nucleus accumbens, either in the medial shell or on the border between the medial shell and the core of the nucleus accumbens. The numbers indicate millimeters from bregma. The brain sections were checked closely for microinjection-induced neurotoxicity. No cell death or gliosis (other than that typically observed following a cannulation) was observed in any group.



DISCUSSION

The present results indicate that neutralizing endogenous NT-3 in the VTA or nucleus accumbens has differential effects on the development of behavioral sensitization to cocaine. Administration of anti-NT-3 into the VTA augmented cocaine-induced behavioral sensitization. In contrast, pretreating the nucleus accumbens with anti-NT-3 enhanced the behavioral-activating effect of cocaine with no further sensitization of the behavioral response over the 4 days of cocaine treatment. Microinjection of a neutralizing antibody for BDNF into either the nucleus accumbens or VTA had no influence on the development of behavioral sensitization to cocaine.

NT-3, BDNF, and cocaine-induced plasticity in the VTA

Dopaminergic neurons in the substantia nigra and VTA express mRNA for BDNF and NT-3 as well as TrkB and TrkC receptors (Ceccatelli et al., 1991; Gall et al., 1992; Timmusk et al., 1993; Seroogy et al., 1994; Numan and Seroogy, 1999). Neurotrophins profoundly influence dopaminergic neurons in the mammalian CNS both during development and adulthood. For example, BDNF promotes the survival and differentiation of developing dopaminergic neurons (Hyman et al., 1991; Akerud et al., 1999) and, in the adult, BDNF

Fig. 2. Intra-accumbal administration of anti-NT-3 enhances the behavioral-activating effect of cocaine but impairs the further development of cocaine-induced behavioral sensitization. Saline, anti-NT-3 (10 or 500 ng), or anti-BDNF (500 ng) were microinjected into the medial nucleus accumbens 10 min prior to cocaine (15 mg/kg, i.p.) on Days 1 through 4. The top panel depicts the total behavioral response recorded over the 120-min period following cocaine administration. Note the significant difference between the behavioral responses on Days 1 and 4 in the saline/cocaine, anti-BDNF/cocaine, and anti-NT-3-10 groups. The middle and bottom panels summarize the time-courses of the behavioral responses recorded on Days 1 and 4, respectively, in the saline/cocaine, anti-BDNF/cocaine, and anti-NT-3-500/cocaine groups. Note the significant differences between the saline/cocaine and anti-NT-3-500/cocaine groups during the 10- and 20-min time periods on both Day 1 (middle panel) and Day 4 (bottom panel). In the top panel, the asterisks represent significant differences between Days 1 and 4 for that treatment group (Fisher's LSD). In the middle and bottom panels, the asterisks represent significant differences from the saline/cocaine group during that time point (Fisher's LSD). There were 7–11 animals per group.

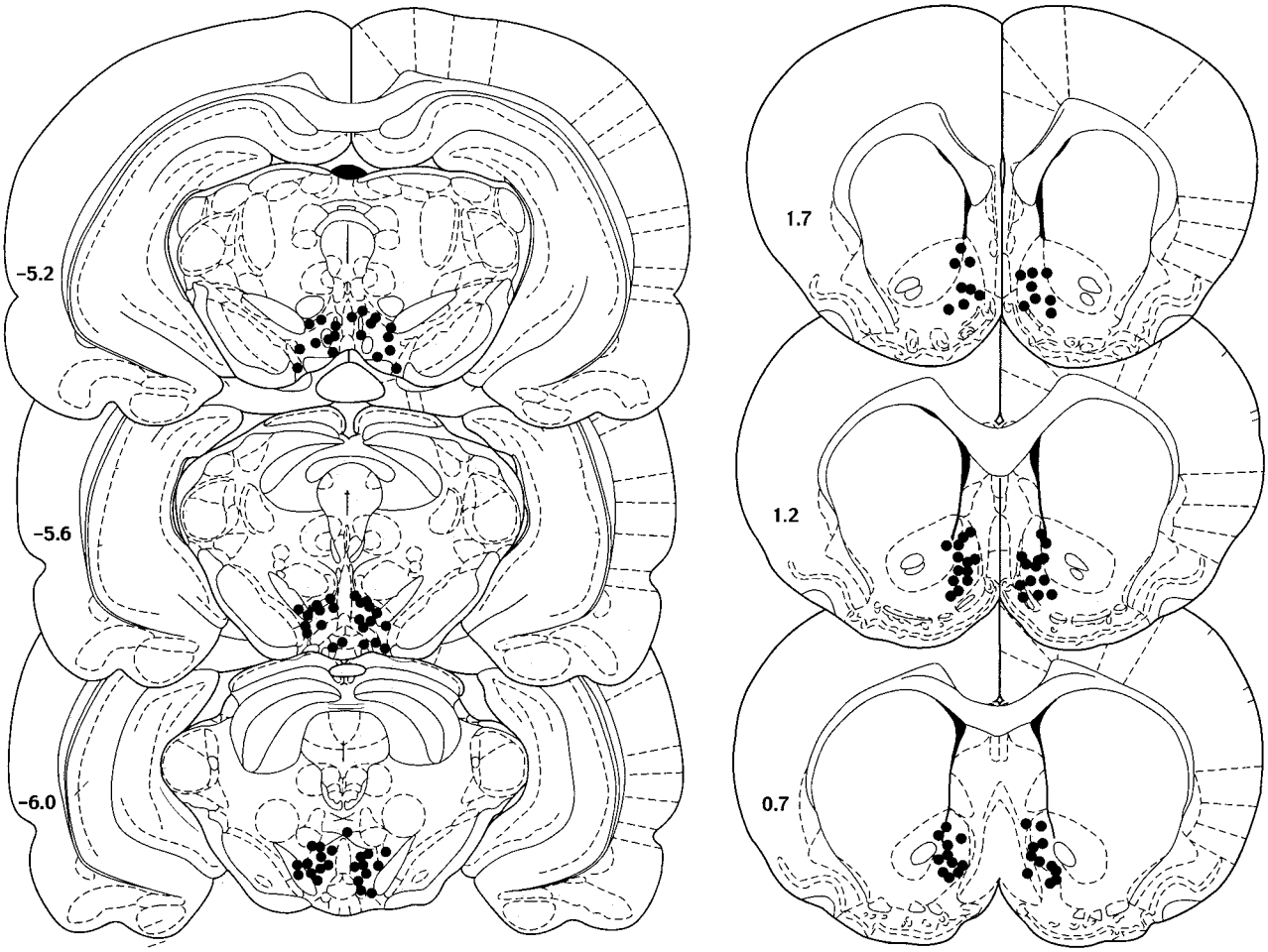


Fig. 3. Location of microinjection sites in the VTA and nucleus accumbens. The filled circles represent the placement of the tips of the microinjection cannulae located in the VTA (left) and nucleus accumbens (right). For the VTA, some of the cannulae tips were located at the border between the VTA and the medial substantia nigra, the ventral medial lemniscus, and the ventral red nucleus. For the nucleus accumbens, all of the cannulae tips were positioned in the medial nucleus accumbens, either in the medial shell or on the border between the medial shell and the core of the nucleus accumbens. The numbers indicate millimeters from bregma.

prevents 1-methyl-4-phenylpyridinium (MPTP)-induced degeneration of dopaminergic neurons (Spina et al., 1992; Frim et al., 1994; Hung and Lee, 1996).

The current results show that neutralizing NT-3 in the VTA augments the initiation of cocaine-induced behavioral sensitization. This result is at odds with recent evidence indicating that repeated microinjections of NT-3 into the VTA cross-sensitize with a subsequent systemic cocaine challenge injection (Pierce et al., 1999). It is unclear, therefore, if endogenous NT-3 contributes to the initiation of behavioral sensitization (Pierce et al., 1999) or impairs this process (present results). It is possible, however, that the neutralization of NT-3 in the present experiments resulted in compensatory processes that enhanced the initiation of behavioral sensitization. For example, a compensatory increase in basic fibroblast growth factor, which is linked to the development of behavioral sensitization (Flores et al., 1998; Flores and Stewart, 2000a,b), or a decrease in glial-derived neurotrophic factor, which impairs the cellular changes in the VTA associated with repeated cocaine injections (Messer et al., 2000), would result in augmented behavioral sensitization. Moreover, it is clear that members of the neurotrophin family are functionally redundant in several ways. Thus, mice heterozygous for a deletion of the NT-3 or BDNF gene have substantial reductions in mRNA for these neurotrophins but survive and appear normal (Korte et al., 1995; Elmer et al., 1997) and different neurotrophins can act through the same receptors and activate the same second-messenger systems (Segal and Greenberg, 1996). Additional biochemical experiments are necessary to define the roles and interactions among neurotrophic factors in the VTA in terms of their respective and collaborative roles in the initiation of behavioral sensitization to cocaine and other psychostimulants.

Although continuous infusion of BDNF into the VTA or substantia nigra enhanced the behavioral hyperactivity induced by an acute injection of amphetamine

(Altar et al., 1992; Martin-Iverson et al., 1994) or cocaine (Horger et al., 1999), an increase in cocaine-induced behavioral hyperactivity was not observed when a withdrawal period was imposed following three daily microinjections of BDNF into the VTA (Pierce et al., 1999). In addition, behavioral sensitization to cocaine was observed in heterozygous BDNF knockout mice (Horger et al., 1999) and the present results indicated that administration of a neutralizing antibody for BDNF into the VTA had no effect on the initiation of behavioral sensitization to cocaine. Based on these results, it is unclear if BDNF-induced plasticity in the VTA contributes to psychostimulant-induced behavioral sensitization.

NT-3, BDNF, and cocaine-induced plasticity in the nucleus accumbens

NT-3 and BDNF are not synthesized in the neostriatum or nucleus accumbens (Ernfors et al., 1990; Maisonpierre et al., 1990; Ceccatelli et al., 1991; Castren et al., 1995; Conner et al., 1997; Furukawa et al., 1998). However, striatal afferent terminals contain NT-3 and BDNF proteins (Conner et al., 1997; Furukawa et al., 1998; Katoh-Semba et al., 1998; Yurek and Fletcher-Turner, 2001), which are transported to the nucleus accumbens and neostriatum anterogradely (Altar and DiStefano, 1998; Reynolds et al., 2000), where they may be released onto medium spiny projection neurons that express TrkB and TrkC receptors (Altar et al., 1994; Fryer et al., 1996; Jung and Bennett, 1996; Numan and Seroogy, 1997; Yan et al., 1997; Canals et al., 1999; Costantini et al., 1999). It is not surprising, therefore, that BDNF and NT-3 have trophic effects in the neostriatum, which include promoting the survival and differentiation of neurons during development (Mizuno et al., 1994; Ventimiglia et al., 1995; Nakao et al., 1996) and protecting striatal neurons from stress- or excitotoxin-induced toxicity (Nakao et al., 1995; Martinez-Serrano and Bjorklund, 1996; Bemelmans et al., 1999).

The present results show that anti-NT-3 administered into the medial shell of the nucleus accumbens increased the behavioral response to cocaine but attenuated the further development of behavioral sensitization to cocaine. Interestingly, intra-accumbal administration of the NT-3 antibody alone had no effect on spontaneous behavioral activity. Consistent with these findings, striatal viral injury, which enhances the behavioral effects of psychostimulants, alters the neurotrophin expression pattern in the striatum (Solbrig et al., 2000). Collectively, these findings suggest that changes in the expression of NT-3 in the basal forebrain may contribute to the development of biochemical, structural, and behavioral changes associated with repeated exposure to cocaine in rats. Consonant with this hypothesis, repeated injections of cocaine increase MAP kinase protein levels in the nucleus accumbens and neostriatum (Valjent et al., 2000). Activation of the MAP kinase signal transduction pathway also influences cocaine-mediated behaviors in rodents. Administration of a MAP kinase kinase inhibitor attenuated cocaine-induced behavioral hyperactivity and place preference (Valjent et al., 2000), which indicates that the MAP kinase signal transduction pathway plays an important role in cocaine-induced behavioral plasticity. There are many substrates for the MAP kinases in both the cytoplasm and nucleus, including other protein kinases, cytoskeletal elements, transcription factors, and tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis (Haycock et al., 1992). The transcription factors activated by the MAP kinases include Elk-1 and, either directly or indirectly, CREB, which has been linked to the regulation of cocaine reward (Segal and Greenberg, 1996; Carlezon et al., 1998; Sgambato et al., 1998; Impey et al., 1999; Vanhoutte et al., 1999). Thus, there are a multitude of cellular processes whereby neurotrophins such as NT-3, acting through MAP kinase, could contribute to cocaine-induced neuronal and behavioral plasticity.

Increases in NT-3 synthesis and release in the nucleus accumbens following repeated psychostimulant injections could contribute to persistent structural changes in this brain region. A sensitizing regimen of amphetamine or cocaine injections as well as cocaine self-administration in rats results in relatively long-lasting increases in dendritic length, the density of dendritic spines, and the number of branched spines in accumbal medium spiny output cells (Robinson and Kolb, 1997, 1999; Robinson et al., 2001). The neurotrophins also contribute to structural changes in the central nervous system through MAP kinase-stimulated upregulation of structural proteins (Kwon and Gurney, 1994; Schnell et al., 1994; Ramirez et al., 1999; Mamounas et al., 2000; Rosenblad et al., 2000). Taken together, these results suggest that increases in the expression of NT-3 protein in the nucleus accumbens and neostriatum may contribute to the structural changes observed in these nuclei following repeated contingent or noncontingent administration of cocaine.

Conclusions

The present results demonstrate that intra-VTA infusion of a neutralizing antibody for NT-3 augments the initiation of behavioral sensitization, whereas neutralizing NT-3 in the nucleus accumbens increases the behavioral response to cocaine but impairs the further development of behavioral sensitization to this psychostimulant. These data are consistent with a growing body of evidence indicating that NT-3 may contribute to biochemical, structural, and behavioral changes in the mesolimbic dopamine system associated with repeated exposure to cocaine.

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