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Endogenous Neurotensin in the Ventral Tegmental Area Contributes to Amphetamine Behavioral Sensitization

Fany Panayi[1,2], Joyce Colussi-Mas[1,2], Laura Lambás-Señas[1,2], Bernard Renaud[1,2], Hélène Scarna[1,2] and Anne Bérod*[,1,2]

[1]INSERM, U512, Lyon, France; [2]Univ Lyon 1, Lab Neuropharmacologie et Neurochimie, Lyon, France

Studies showing psychostimulant-like effects of exogenous neurotensin (NT) infused into the ventral tegmental area (VTA) prompted us to examine the role in the VTA of the endogenous NT in behavioral sensitization to amphetamine. Rats were sensitized to amphetamine by means of a subcutaneous amphetamine (1 mg/kg) injection, and the same dose was injected 7 days later to evaluate the expression of sensitization. The highly selective NT-receptor antagonist SR 142948A was injected into the VTA prior to the first and/or second amphetamine administration. SR 142948A (5 pmol/side) given before the first amphetamine exposure prevented the induction of behavioral sensitization, but did not alter the acute response to amphetamine. SR 142948A given with the second amphetamine administration did not affect the expression of behavioral sensitization. In contrast to administration into the VTA, intraperitoneal administration of SR 142948A (0.03, 0.1, or 0.3 mg/kg) had no detectable effect on the induction of amphetamine sensitization. These results suggest that activation of VTA NT receptors by endogenous NT may contribute to the neuroadaptations underlying behavioral sensitization to amphetamine.
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INTRODUCTION

Administration of psychostimulants such as amphetamine and cocaine leads to enduring augmentation of the behavioral effects of these drugs, a phenomenon known as sensitization. Sensitization develops in lockstep with drug craving, and it has been suggested that the neurophysiological adaptations underlying behavioral sensitization may be among the mechanisms driving addictive behavior (for reviews, see Robinson and Berridge, 1993; Vezina, 2004).

The mesolimbic dopamine (DA) pathway, which originates in the ventral tegmental area (VTA) and projects to regions that include the nucleus accumbens (NAC), is considered a fundamental component of the neural system that mediates the effects of drugs of abuse. Compelling evidence indicates that induction of behavioral sensitization to amphetamine occurs chiefly in the VTA, whereas expression is promoted by the NAC. Repeated amphetamine injections into the VTA are sufficient to sensitize rats to a subsequent amphetamine injection given systemically or into the NAC (Kalivas and Weber, 1988; Hooks *et al*, 1992; Perugini and Vezina, 1994; Cador *et al*, 1995). In contrast, injection of amphetamine into the NAC stimulates locomotion, but when repeated fails to induce behavioral sensitization (Hooks *et al*, 1992; Cador *et al*, 1995).

Although DA plays a crucial role in behavioral sensitization, the behavioral alterations induced by psychostimulants result from a complex process that involves interactions among several neurotransmitters, most notably the excitatory amino acid glutamate (reviewed by Vanderschuren and Kalivas, 2000), neurotrophic factors (Pierce and Bari, 2001), neuropeptides such as CCK (Beinfeld, 2003), opioids (Magendzo and Bustos, 2003), CART peptides (Jaworski *et al*, 2003), and neurotensin (NT) (Bérod and Rostène, 2002). All these peptides are closely related to the midbrain DA systems and their regulation (Mavridis and Besson, 1999; Binder *et al*, 2001b; Rotzinger *et al*, 2002; Jaworski *et al*, 2003). NT has been extensively studied, and although traditionally viewed as an endogenous neuroleptic (Kinkead and Nemeroff, 2002), this peptide is also emerging as a possible mediator of psychostimulant effects (Bérod and Rostène, 2002).

Psychostimulant administration induces the co-release of NT and DA in the NAC and medial prefrontal cortex (PFC) (During *et al*, 1992; Hertel *et al*, 1996; Gruber *et al*, 2002). When injected into the VTA, NT produces effects similar to those of systemically injected psychostimulants, such as increased motor activity (Kalivas *et al*, 1983) and elevated

*Correspondence: Dr A Bérod, INSERM, U512, Université Claude Bernard, Faculté de Pharmacie, 8 Avenue Rockefeller, 69373 Lyon Cedex 08, France, Tel: +33 4 78 77 75 53, Fax: +33 4 78 77 72 09, E-mail: aberod@sante.univ-lyon1.fr
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extracellular DA levels in the NAC (Kalivas and Duffy, 1990; Laitinen *et al*, 1990; Sotty *et al*, 1998). However, when injected into the NAC, NT has an opposite effect on psychostimulant-induced behaviors, attenuating the locomotion produced by systemic amphetamine or cocaine (Ervin *et al*, 1981; Robledo *et al*, 1993; Steinberg *et al*, 1994). Altogether, these results suggest that two NT pathways with opposite effects, projecting to the VTA or to the NAC, may facilitate or attenuate the behavioral effects of psychostimulants, respectively. The net effect of these two NT pathways when the brain is challenged by psychostimulants needs to be determined. Recent studies in rats found that repeated systemic administration of specific NT-receptor antagonist had a net inhibitory influence on amphetamine- and cocaine-induced behavioral activation (Horger *et al*, 1994; Betancur *et al*, 1998a; Rompré and Perron, 2000; Panayi *et al*, 2002). This result supports a role for endogenous NT in the behavioral effects of psychostimulants, but does not provide information about the NT systems affected by psychostimulants.

The psychostimulant-like effects of NT in the VTA and the key role of VTA DA neurons in mediating the stimulant effects of amphetamine prompted us to hypothesize that NT receptors located in the VTA contribute to amphetamine-induced behaviors. The primary purpose of the present study was to investigate this hypothesis. To this end, rats were sensitized by a single subcutaneous (s.c.) injection of amphetamine (1 mg/kg), then challenged with the same s.c. dose 7 days later (Robinson *et al*, 1982; Vanderschuren *et al*, 1999). Behavioral sensitization to amphetamine was evaluated following administration of the highly selective NT-receptor antagonist SR 142948A into the VTA at induction (first amphetamine injection) and/or expression (second amphetamine injection) of sensitization.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Harlan, France) weighing 200–220 g on arrival were used. Rats for experiments 1 and 2 were housed in pairs before surgery and individually after surgery; those for experiment 3 were housed four per cage. They were maintained in a temperature-controlled environment (22°C) with a 12 h light/dark cycle (lights on at 07:00 h) and free access to food and water. Rats were allowed to habituate 1 week to the animal room prior to their use. All procedures were carried out in accordance with the European Community Council Directives for the care and use of laboratory animals (86/609/EEC).

Drugs

D-amphetamine sulfate (Sigma-Aldrich, France) was dissolved in 0.9% saline and injected s.c. (1 ml/kg) in the dose of 1 mg/kg. NT (Sigma) was dissolved in phosphate buffer saline (PBS, 150 mM NaCl, 2.8 mM KCl in 2.8 mM sodium phosphate buffer, pH 7.4) in a concentration of 6×10^{-3} M, and aliquots were stored at −30°C. Final solutions were freshly prepared in PBS. For intracerebral infusions, the NT-receptor antagonist SR 142948A (Sanofi-Synthélabo, Montpellier, France) was freshly dissolved in dimethylsulfoxide at 50°C and diluted in PBS to obtain a 10^{-5} M solution (final concentration of dimethylsulfoxide, 0.1%). For systemic administrations, SR 142948A was suspended with Tween 80 in saline and injected intraperitoneally (i.p.; 1 ml/kg) in a dose of 0.03, 0.1, or 0.3 mg/kg.

Surgery and Implantation of Cannulae

For studies requiring intracerebral infusions, 1 week after their arrival, rats were anesthetized with i.p. chloral hydrate (400 mg/kg) and placed in a stereotaxic apparatus (David Kopf, Tunjunga, CA, USA). A stainless steel guide cannula (23 gauge) was implanted with the tip 1 mm above the VTA on each side. Cannulae were angled at 10° to the vertical, and stereotaxic coordinates according to the atlas of Paxinos and Watson (1998) were A/P, −5.8 mm from the bregma; L, ±2.2 mm from the midline; and D/V, −7.5 mm from the skull surface. Stainless steel obturators (30 gauge) were placed into the guide cannulae. The rats were allowed at least 7 days to recover from surgery, during which they were handled and weighed daily.

Behavioral Measurements

Motor activity was monitored in Plexiglas activity cages ($26 \times 41 \times 20$ cm) equipped with an array of four parallel horizontal infrared beams, two at the front and two at the back, positioned 4 cm above the floor to measure horizontal activity. The activity cages were linked to a computer that recorded photocell beam breaks. Locomotion was estimated by determination of successive breaks at the front and at the back and *vice versa* (crossovers). The number of crossovers was continuously recorded and cumulated over 10-min intervals.

On test days, the rats were habituated to the activity cages for 2 h before treatment.

For injection into the VTA, the rats were gently restrained, and each guide cannula obturator was replaced by an injection cannula (30 gauge) extending 1 mm below the tip of the guide cannula. Simultaneous bilateral injections (0.5 µl/side) were given over 50 s using an infusion pump, and the injection cannulae were left in place for an additional 50 s to allow the compound to diffuse away from the tips of the cannulae. After 1 min, the rats received either a second microinjection into the VTA (NT or its vehicle; experiment 1) or a s.c. injection (amphetamine or saline; experiment 2). The obturators were substituted for the injection cannulae at the end of the intra-VTA injections. All rats were returned to the activity cages, and locomotion was recorded for 2 h.

Systemically treated rats were injected with SR 142948A or vehicle, followed 1 h later by administration of amphetamine. Following injections, each rat was immediately returned to its activity cage. Locomotor activity was recorded during 2 h after the amphetamine injection.

All experiments were performed between 10:00 and 15:00 h.

Experimental Design

In experiment 1, as a control experiment, we examined the effect of intra-VTA administration of SR 142948A on the

locomotor activity induced by NT injection into the VTA. Rats received four pairs of microinjections, in random order, with at least 72 h between pairs. The microinjections, with the doses reported as the amount/per side, consisted of (1) vehicle + PBS, (2) SR 142948A (5 pmol) + PBS, (3) vehicle + NT (1.5 nmol) and (4) SR 142948A (5 pmol) + NT (1.5 nmol). The doses of NT and NT-receptor antagonist were selected based on preliminary data from our laboratory and on published data (Kalivas *et al*, 1983; Leonetti *et al*, 2002).

In experiment 2, we sought to determine whether NT receptors in the VTA played a functional role in behavioral sensitization to amphetamine. On day 1, rats received a bilateral microinjection of vehicle or SR 142948A into the VTA, followed 1 min later by a systemic injection of saline or amphetamine (1 mg/kg, sc). This yielded four groups based on the day 1 injection: vehicle + saline, SR 142948A + saline, vehicle + amphetamine and SR 142948A + amphetamine. After 7 days, the rats pretreated with amphetamine received a second bilateral injection into the VTA, of SR 142948A or its vehicle, prior to the challenge injection of amphetamine (1 mg/kg, s.c. on day 8). Thus, amphetamine sensitization was assessed in four groups treated as follows: (1) vehicle pretreatment on days 1 and 8 (controls), (2) SR 142948A pretreatment on day 1 (NT-receptor blockade during induction of amphetamine sensitization), (3) SR 142948A pretreatment on day 8 (NT-receptor blockade during expression of sensitization), or (4) SR 142948A pretreatment on days 1 and 8 (NT-receptor blockade during induction and expression). Each animal was used only once.

The purpose of experiment 3 was to test the effect of systemic NT-receptor blockade on the induction of behavioral sensitization to amphetamine. Rats were sensitized, as in the previous experiment, by a single amphetamine injection (1 mg/kg s.c. on day 1) and challenged with the same amphetamine dose 7 days later (day 8). On day 1, these rats were pretreated with vehicle or SR 142948A (0.03, 0.1, or 0.3 mg/kg i.p.), whereas on day 8 all rats were pretreated with vehicle.

Assessment of Cannulae Placement

At completion of the experiments, the animals that had been implanted with guide cannulae were overdosed with sodium pentobarbital (Sanofi). The brain of each rat was removed, frozen, and stored at −40°C. Coronal sections (60 μm thick) were cut on a cryostat, mounted on slides, and stained with neutral red for evaluation of cannulae position.

Tyrosine hydroxylase (TH) immunohistochemistry was performed on a few rat brains to examine the viability of DA neurons near the tips of the cannulae. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and perfused intracardially with saline and freshly prepared 4% paraformaldehyde solution in 0.1 M phosphate buffer (PB), pH 7.4. The brains were then rapidly removed, placed in fresh fixative overnight, cryoprotected in 0.1 M PB containing 30% sucrose at 4°C, and frozen for 3 min in isopentane at −50°C.

Coronal sections (30 μm thick) were cut on a freezing microtome along the entire VTA and collected into four adjacent series of sections. Sections were immersed in a solution containing 50 mM PB, pH 7.4, 30% ethylene glycol, 20% glycerol, then stored at −20°C for subsequent processing. Immunohistochemistry was performed according to the avidin-biotinylated horseradish peroxidase complex method with the Elite ABC kit (Vector Laboratories, Burlingame, CA). Briefly, free-floating sections were washed with PBS (pH 7.4) (3 × 10 min) and incubated 48 h at 4°C with the primary TH antibody (Institut Jacques Boy, Reims, France) diluted 1/32 000 in PBS containing 0.3% triton X-100 (PBST) and 1% bovine serum albumin. After three rinses in PBST, the sections were incubated for 90 min at room temperature with biotinylated goat anti-rabbit diluted 1/1000 in PBST, then for 60 min with preformed avidin-biotinylated horseradish peroxidase complex diluted 1/100 in PBST. Visualization of bound peroxidase was achieved by reaction in a solution of 50 mM Tris–HCl, pH 7.6, containing 0.02% 3-3′-diaminobenzidine, 0.8% nickel chloride, and 0.003% H_2O_2. The reaction was stopped by three washes with PBST.

Statistical Analysis

In experiment 1, locomotor activity data (total crossovers) were analyzed using two-way analysis of variance (ANOVA) with repeated measures over treatments, followed by the Student Newman–Keuls test for multiple comparisons. In experiment 2, time-course data (crossovers /10 min) recorded on day 1 were subjected to two-way ANOVA with one within-subject factor (time) and one between-subject factor (treatment). *Post hoc* comparisons were made using the Student Newman–Keuls test. In experiments 2 and 3, sensitization was assessed by comparing amphetamine-induced locomotion on days 1 and 8. Time-course data were subjected to ANOVA with two within-subject factors (time and treatment day). The cumulated crossovers recorded during the 90 min following amphetamine administration were analyzed with paired *t*-tests.

RESULTS

Histological Analysis

As illustrated in Figure 1, the injection cannulae were placed within the main population of DA neurons in the VTA, as shown by their tracks in relation to TH-immunostained neurons. Although mild compression of DA neurons and gliosis were observed adjacent to the cannula tips, damage to the surrounding regions was minimal. Of the 69 rats implanted in this study, 53 had both injection cannulae implanted within the rostrocaudal and mediolateral VTA (including the parabrachialis pigmented nucleus and the paranigralis nucleus) and were therefore used for the statistical analysis.

Intra-VTA Administration of SR 142948A Reversed the Locomotor-activating Effect of Intra-VTA Administration of NT (Experiment 1)

The data in Figure 2 summarize the effects of SR 142948A or its vehicle on locomotor activity induced by NT or its vehicle (PBS). Two-way ANOVA with repeated measures revealed significant main effects of NT ($F_{1,9} = 8.71$, $p < 0.05$)

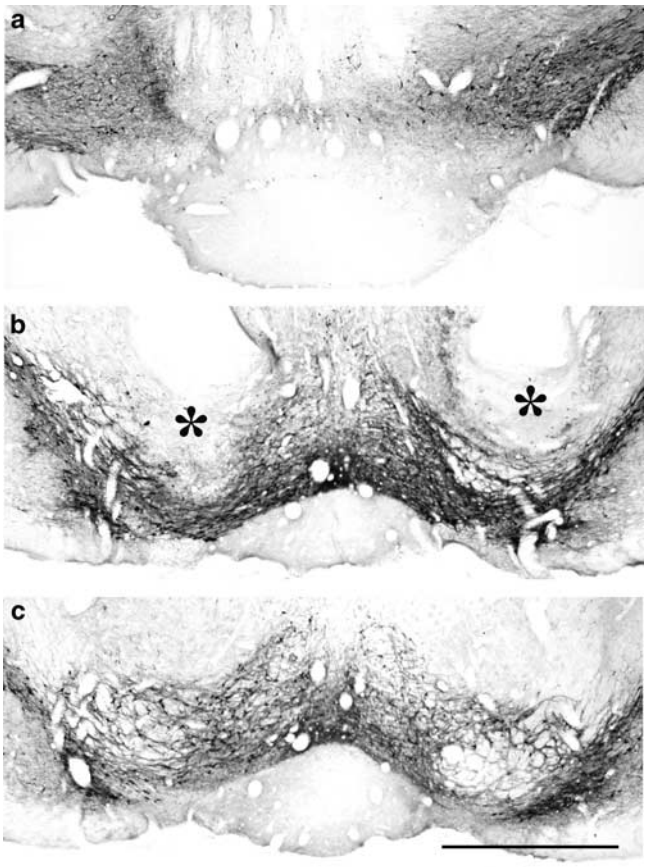

Figure 1 Position of the VTA injection cannulae relative to the zone where DA cell bodies are found. Photomicrographs show coronal sections through the VTA ((a) anterior VTA; (b) median VTA; (c) posterior VTA), with the location of the bilateral guide cannulae and injection tracks. Black asterisks indicate the injection cannula tips. The sections are typical of implantations observed in the animals used in this study. Scale bar: 1 mm.

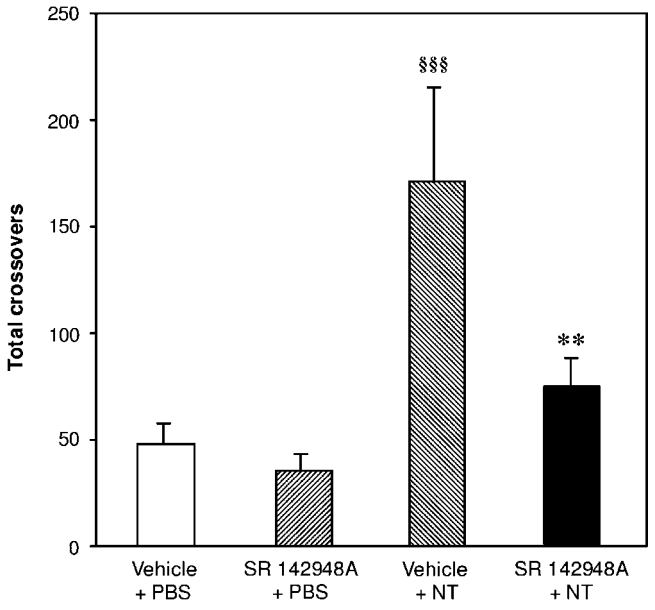

Figure 2 Experiment 1: Prevention of NT-induced locomotion by pretreatment with SR 142948A (5 pmol/0.5 μl) into the VTA. The data represent the mean (+SEM) locomotor activities (crossovers) scored over the 2-h post-microinjection period ($n=10$). $^{§§§}p<0.001$ compared with vehicle + PBS and with SR 142948 + PBS, $^{**}p<0.01$ compared with vehicle + NT (Newman–Keuls *post hoc* test).

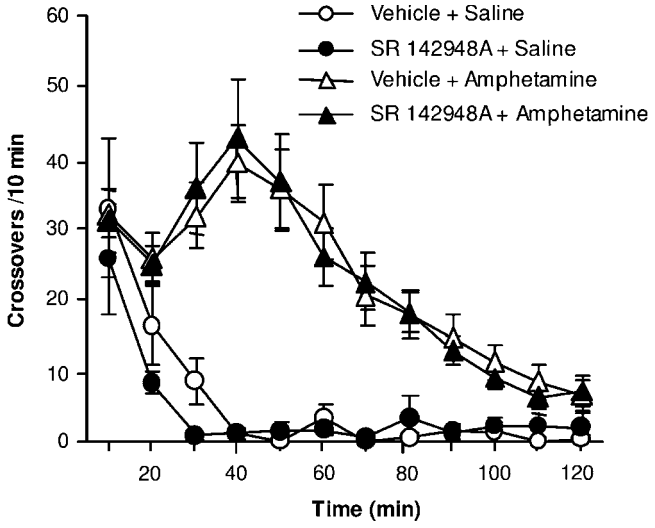

Figure 3 Experiment 2: Effect of intra-VTA administration of SR 142948A (5 pmol/0.5 μl) on locomotor activity induced by the first exposure to amphetamine (1 mg/kg, s.c.) or saline. The data represent the mean (±SEM) locomotor activity scores (crossovers), summed across 10-min intervals, over the 120 min following the amphetamine or saline injection. There were six rats per group for controls (Vehicle + Saline and SR 142948A + Saline), 15 rats for Vehicle + Amphetamine group and 16 for SR 142948A + Amphetamine group. The two amphetamine groups are significantly different from the two saline groups ($p<0.01$ for each of the four comparisons).

and SR 142948A ($F_{1,9}=6.70$, $p<0.05$), as well as a significant interaction between these treatments ($F_{1,9}=5.58$, $p<0.05$). *Post hoc* multiple comparisons showed that bilateral microinjection of NT into the VTA induced a significant increase in locomotion (vehicle + NT *vs* vehicle + PBS or SR 142948A + PBS, $p<0.001$) that was significantly attenuated by SR 142948A (SR 142948A + NT *vs* vehicle + NT, $p<0.01$). On the other hand, this NT-receptor antagonist did not decrease the locomotor activity induced by the infusion procedure (SR 142948A + PBS *vs* vehicle + PBS, $p=0.69$). Likewise, there is no significant statistical difference between the locomotor activity exhibited by rats following SR 142948A + PBS or SR 142948A + NT treatments ($p=0.23$). These results showed that intra-VTA SR 142948A at a dose of 10^{-5} M blocked the locomotor-activating effect of NT in the VTA.

Intra-VTA Administration of SR 142948A had no Effect on the Acute Behavioral Responses to Amphetamine (Experiment 2)

Figure 3 shows the effect of bilateral intra-VTA injection of SR 142948A or its vehicle on the locomotor activity induced by the first amphetamine or saline injection (day 1). The overall ANOVA indicated significant main effects of treatment ($F_{3,39}=8.51$, $p<0.001$) and time ($F_{11,429}=21.81$, $p<0.0001$), as well as a significant interaction between these

factors ($F_{33,429} = 4.51$, $p < 0.0001$). *Post hoc* multiple comparisons revealed that the vehicle + amphetamine and SR 142948A + amphetamine groups were significantly different from the vehicle + saline and SR 142948A + saline groups ($p < 0.01$ for each comparison). Moreover, bilateral injection of SR 142948A into the VTA had no significant effect on acute amphetamine-induced locomotion (SR 142948A + amphetamine *vs* vehicle + amphetamine groups) or on basal locomotion (SR 142948A + saline *vs* vehicle + saline groups).

Intra-VTA Administration of SR 142948A Altered the Induction but not the Expression of Behavioral Sensitization to Amphetamine (Experiment 2, Figure 4)

To determine whether endogenous NT contributed to the induction or expression of behavioral sensitization, amphetamine-treated rats on day 1 received, 7 days later, intra-VTA injections of SR 142948A or its vehicle, followed by an amphetamine challenge (day 8). Locomotor activities recorded following the first (day 1) and the second (day 8) amphetamine injections are shown in Figure 4.

Figure 4a shows the data from animals pretreated with vehicle on days 1 and 8 and subsequently given amphetamine. Analysis of the time-course data revealed significant main effects of treatment day ($F_{1,7} = 125.72$, $p < 0.0001$) and time ($F_{11,77} = 23.93$, $p < 0.0001$), as well as a significant interaction between these factors ($F_{11,77} = 9.37$, $p < 0.0001$). Analysis of cumulated data indicated a significant difference between the behavioral responses on days 1 and 8 ($t_7 = 11.20$, $p < 0.0001$).

Figure 4b reports the data from animals given SR 142948A before only the first amphetamine injection. The time-course data analysis showed a significant main effect of time ($F_{11,66} = 11.03$, $p < 0.0001$) but not treatment day ($F_{1,6} = 1.55$, $p = 0.26$), with no significant interaction between these factors ($F_{11,66} = 0.72$, $p = 0.72$). Analysis of the cumulated data found no significant difference between days 1 and 8 ($t_6 = 0.98$, $p = 0.40$), that is, that the sensitization was prevented.

Figure 4c depicts the data from rats pretreated with SR 142948A on day 8 only, prior to the challenge amphetamine injection. The time-course data analysis indicated significant main effects of treatment day ($F_{1,7} = 18.00$, $p < 0.01$) and time ($F_{11,77} = 16.85$, $p < 0.0001$), as well as a significant interaction between these factors ($F_{11,77} = 11.83$, $p < 0.0001$). The paired *t*-test performed on the cumulated data indicated a significant difference between the behavioral responses on days 1 and 8 ($t_7 = 4.34$, $p < 0.01$).

Finally, Figure 4d shows the data from rats pretreated with SR 142948A both on days 1 and 8 prior to each amphetamine injection. The time-course data analysis

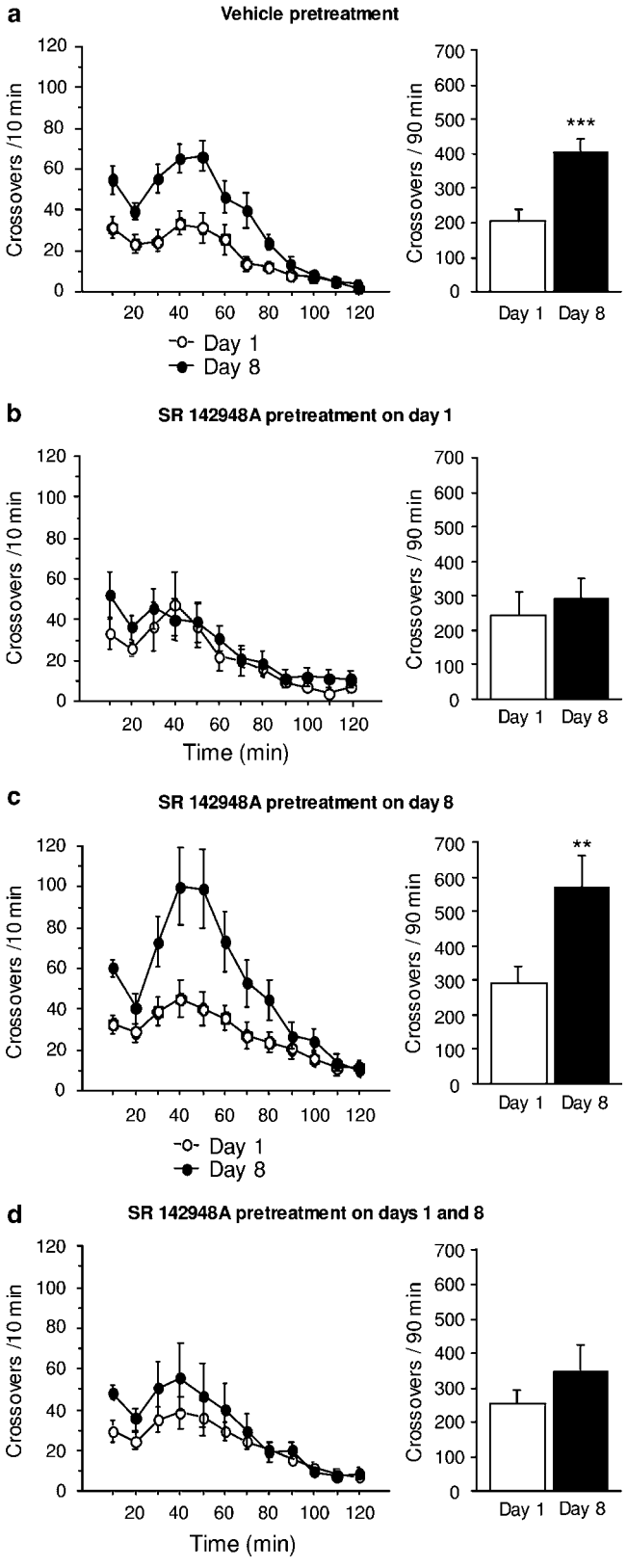

Figure 4 Experiment 2: Effect of intra-VTA administration of SR 142948A on locomotor sensitization to amphetamine. On days 1 and 8, rats received bilateral microinjections of vehicle or SR 142948A (5 pmol/0.5 µl) into the VTA, then, 1 min later, a systemic injection of amphetamine (1 mg/kg, s.c.). Behavior was monitored for the following 2 h. There were seven rats in groups pretreated with SR 142948A at day 1, and eight rats in the three other groups. For each of four experimental groups, the data depicted in the left-hand panels reflect the time-course of crossovers (mean ± SEM) counted after amphetamine administration. The data in the right-hand panels are the total crossovers (mean + SEM) cumulated during the first 90 min following amphetamine administration on days 1 and 8. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with day 1.

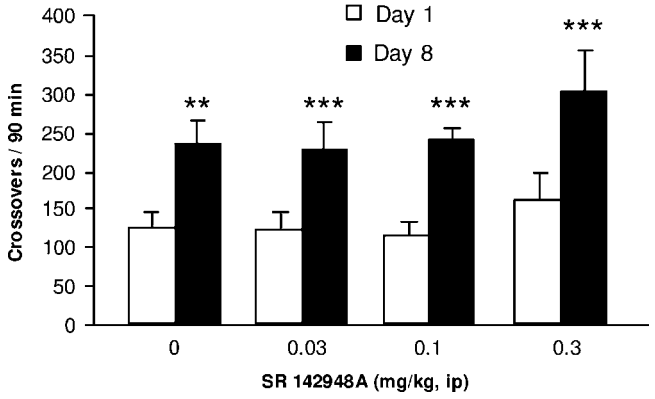

Figure 5 Experiment 3: Effect of systemic administration of SR 142948A on the induction of locomotor sensitization to amphetamine. The locomotor responses to amphetamine ($n = 10$ for each group) were determined on days 1 and 8 in rats pretreated with SR 142948A on day 1. Data are expressed as mean + SEM crossovers cumulated during the 90-min period after amphetamine administration. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with day 1.

showed no significant main effect of treatment day ($F_{1,7} = 1.51$, $p = 0.26$), but found a significant main effect of time ($F_{11,77} = 12.04$, $p < 0.0001$); there was no significant interaction between these factors ($F_{11,77} = 1.57$, $p = 0.12$). Analysis of the cumulated data indicated no significant difference between the behavioral response on days 1 and 8 ($t_7 = 1.33$, $p = 0.22$).

Taken in concert, these data show that NT-receptor blockade in the VTA prevents the induction but not the expression of locomotor sensitization to amphetamine.

Systemic Administration of SR 142948A did not Alter the Induction of Behavioral Sensitization to Amphetamine (Experiment 3)

Figure 5 summarizes the locomotor activity recorded over the first 90 min following amphetamine injection to rats pretreated with a systemic injection of vehicle or of SR 142948A 0.03, 0.1, or 0.3 mg/kg prior to the first amphetamine injection. These data were analyzed with separate paired *t*-tests, which were planned comparisons between days 1 and 8 within treatment groups. These analyses revealed significant differences between days 1 and 8 in all groups: vehicle group ($t_9 = 4.49$, $p < 0.01$), SR 142948A 0.03 mg/kg group ($t_9 = 6.42$, $p < 0.001$), SR 142948A 0.1 mg/kg group ($t_9 = 5.75$, $p < 0.001$), and SR 142948A 0.3 mg/kg group ($t_9 = 6.07$, $p < 0.001$). Thus, behavioral sensitization occurred both in the rats given the vehicle and in those given systemic SR 142948A.

DISCUSSION

We examined the role of NT receptors in the induction and expression of behavioral sensitization to amphetamine. Injection of the NT-receptor antagonist SR 142948A into the VTA markedly attenuates the induction of behavioral sensitization to amphetamine, but had no effect on either the expression of sensitization or the acute locomotor response to amphetamine. These findings suggest that NT-receptor activation in the VTA after amphetamine injection may contribute to the long-term amphetamine-induced neuronal plasticity that underlies behavioral sensitization. In contrast, when administered systemically, SR 142948A did not affect the induction of behavioral sensitization to amphetamine. This suggests that effects of SR 142948A on other NT-receptor-expressing brain regions may counteract the impairment of amphetamine behavioral sensitization related to VTA NT-receptor blockade.

These results provide valuable understanding into the role of NT transmission in behavioral sensitization to amphetamine. Whereas previous studies demonstrated a permissive role of endogenous NT in the behavior-activating effects of amphetamine or cocaine, they did not directly seek to identify the NT systems involved in the acute and/or long-term effects of these psychostimulants (Horger *et al*, 1994; Betancur *et al*, 1998a; Rompré and Perron, 2000; Costa *et al*, 2001; Panayi *et al*, 2002). Our finding that intra-VTA injection of SR 142948A, an antagonist of the two G-protein-coupled NT receptors NT1 and NT2 (Gully *et al*, 1997), decreases the long-term effect of a single amphetamine injection is consistent with a release of NT in the VTA and an involvement of NT in the neuroadaptations that underlie behavioral sensitization.

The behavioral sensitization induced by psychostimulants depends on adaptations occurring in the dopaminergic mesolimbic system, and its induction is largely due to interactions between DA and other neurotransmitters in the VTA. NT and its two receptors NT1 and NT2 are expressed at high levels in the VTA. NT is present in cell bodies and dendrites that also contain DA, as well as in a dense network of non-DA nerve terminals deriving from extrinsic sources (Bayer *et al*, 1991; Woulfe and Beaudet, 1992; Zahm *et al*, 2001). NT-immunoreactive dense core vesicles are visible near segments of somatodendritic and nerve terminal plasma membrane, suggesting that NT may be released from dendrites as well as from nerve terminals (Bayer *et al*, 1991). The NT1 receptor is found chiefly in DA midbrain neurons, but a small proportion is associated with non-DA axons and astroglial cells (Nicot *et al*, 1995; Boudin *et al*, 1998). Although NT2-receptor expression on glial cells has been shown by *in situ* hybridization (Sarret *et al*, 1998; Walker *et al*, 1998), immunohistochemistry studies suggest a strong association of NT2 receptor with neurons, and predominantly with their dendritic arbors (Sarret *et al*, 2003). Several evidence indicate that NT receptors on glial cells, like NT receptors on neurons, are functional (Hösli *et al*, 1995; Nouel *et al*, 1999; Trudeau, 2000). Thus, when released in the VTA, NT is in a position to act on DA cell bodies, as well as on axon terminals and on non-neuronal cells.

At present, there is no direct evidence to support a link between the long-term effect of NT-receptor activation on amphetamine-induced behavioral plasticity and alterations in DA function. However, the primary effect of NT in the VTA is to increase DA-neuron-firing activity (Sotty *et al*, 1998; Werkman *et al*, 2000), and *in vitro* this effect is linked to a sustained increase in intracellular calcium levels (St-Gelais *et al*, 2004). Given that calcium signaling in the VTA plays a critical role in the induction of behavioral sensitization (reviewed by Licata and Pierce, 2003), we

suggest that an NT-induced elevation in calcium levels in DA neurons may be among the mechanisms underlying the effects of NT on sensitization to amphetamine. Indeed, there are numerous cellular and nuclear targets of calcium, including calcium/calmodulin-dependent protein kinase, mitogen-activated protein kinases, and the transcription factors CREB that are relevant for the induction of sensitization (reviewed by Licata and Pierce, 2003). Although the molecular identity of the NT receptor(s) involved in intracellular calcium elevation remains to be established, we suggest that the NT1 receptor may play a major role. The NT1 receptor is highly expressed in DA neurons in the VTA and, in addition, NT2-receptor activation does not cause calcium elevation in cultured cerebellar neurons expressing only NT2 receptors (Sarret *et al*, 2002). In addition to its effects on DA neurons, NT may act on NT receptors located on axonal terminals to ensure presynaptic regulation of the release of neurotransmitters involved in sensitization. The presence of cell bodies expressing NT receptors in the PFC (Boudin *et al*, 1996; Alexander and Leeman, 1998), which is a major source of excitatory drive to the VTA, suggests that the NT-receptor-containing terminals in this area may belong to the glutamatergic PFC–VTA pathway. Therefore, NT may also contribute to the induction of amphetamine sensitization via stimulation of the glutamatergic output from the PFC, which plays a crucial role in this phenomenon (reviewed by Wolf, 1998; Vanderschuren and Kalivas, 2000).

It is noteworthy that injecting the NT antagonist into the VTA prior to the amphetamine injection blocked the development of behavioral sensitization but did not alter the acute behavioral response to amphetamine. This absence of an acute effect is probably linked to the mechanisms of amphetamine action. Indeed, the behavioral effects of amphetamine are largely dependent on an elevation in extracellular DA levels in the NAC, related to a direct effect on DA transporter function (reviewed by White and Kalivas, 1998). This transporter-mediated DA release, which is independent of impulse flow (Westerink *et al*, 1987; Carboni *et al*, 1989), strongly activates a DA inhibitory feedback mechanism and masks non-DA excitatory effects on mesolimbic DA neurons (Shi *et al*, 2000). Thus, amphetamine-induced DA release bypasses additional control by VTA inputs, so that the VTA NT-stimulating effect on DA release in the NAC (Laitinen *et al*, 1990; Sotty *et al*, 1998) and on locomotor activity (Bauco and Rompré, 2003) might be ineffective under amphetamine exposure. Altogether, our results suggest that NT-receptor activation within the VTA may promote intracellular events that appear insufficient to affect the acute response to amphetamine but may lead or contribute in the long-term to behavioral sensitization.

In rats pre-exposed to amphetamine, we found that intra-VTA injection of SR 142948A prior to the amphetamine challenge failed to impair the expression of behavioral sensitization. This finding is in line with the general concept that VTA is an anatomical substrate for induction, but not expression, of amphetamine sensitization. Given that the NAC plays a critical role in the expression of behavioral sensitization, a role for NT in the NAC may be worth examining since NT is released in this structure after amphetamine (Gruber *et al*, 2002) and since exogenous NT exerts inhibitory effects on acute psychostimulant-induced hyperlocomotion (Ervin *et al*, 1981; Robledo *et al*, 1993; Steinberg *et al*, 1994).

Finally, we found that systemic blockade of NT receptors with SR 142948A does not prevent the induction of behavioral sensitization to amphetamine. These data are in line with our previous results (Panayi *et al*, 2002) using systemic SR 48692, another NT antagonist, and showing that a sustained blockade of NT receptors throughout the amphetamine regimen (i.e. during induction and expression phases) was required to reduce amphetamine behavioral sensitization. They, however, differ from those obtained by Rompré and Perron (2000), who reported a decrease of amphetamine sensitization when SR 48692 was systemically administered during the induction phase. Such a discrepancy could be due to methodological variables, since in this latter study amphetamine-induced locomotion was recorded without prior habituation of the animals to the test cage and with the lights off, two conditions that by themselves stimulate locomotion. On the other hand, it may be argued that cerebral SR 142948A levels achieved after a systemic injection are too low to ensure complete NT-receptor blockade in the VTA. This explanation is unlikely, however, because SR 142948A crosses the blood–brain barrier and its systemic administration in doses lower than those used in this study antagonizes several effects induced by exogenous NT administration into the brain (Gully *et al*, 1997). Moreover, systemic administration of this NT antagonist has been used to demonstrate the effect of disrupting of brain NT transmission on DA efflux in the NAC (Brun *et al*, 2001), behaviors (Binder *et al*, 2001a, 2002; Casti *et al*, 2004), and expression of transcription factors (Binder *et al*, 2004). An alternative explanation is that regions other than the VTA may intervene in the development of behavioral sensitization to amphetamine. This interpretation is consistent with the multiplicity of neuroanatomical sites where NT release occurs after amphetamine exposure, such as the NAC (Gruber *et al*, 2002), the PFC (During *et al*, 1992; Hertel *et al*, 1996), and the VTA (as suggested by the present study), and with the broad distribution of NT receptors sensitive to SR 142948A in the brain (Betancur *et al*, 1998b). It is also congruent with the fact that NT has opposite effects on locomotion depending on its site of injection (see introduction). In this regard, the presence of NT nerve terminals and receptors in the PFC, NAC, and amygdala, all of which are involved in various aspects of amphetamine sensitization (reviewed by Vanderschuren and Kalivas, 2000), could support different functions for NT in this phenomenon. Whatever the brain regions involved, our results indicate that blocking all NT receptors in the brain may mask the behavioral effect of blocking only the NT receptors in the VTA.

In conclusion, the present experiments suggest that NT may be among the VTA neurotransmitters that contribute to induce behavioral sensitization to amphetamine. They emphasize the importance of peptidergic systems in the complex processes involved in the neuronal plasticity that underlies long-term behavioral sensitization, and they provide new insights into the cellular effects of a drug of abuse.

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