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Blockade of neurokinin-3 receptors modulates dopamine-mediated behavioral hyperactivity

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ABSTRACT

Acute activation or blockade of neurokinin-3 (NK-3) receptors has been shown to alter dopamine-mediated function and behaviors, however long-term effects of NK-3 receptor blockade remain largely unknown. The present study investigated whether acute and repeated administration of the NK-3 receptor antagonist SB 222200 altered hyperactivity induced by cocaine, and examined its effects on dopamine D1 receptor density in the striatum. Adult male CD-1 mice received either vehicle or SB 222200 (2.5 or 5 mg/kg, s.c.) 30 min before a cocaine injection (20 mg/kg, i.p.) and behavioral responses were recorded. Mice that were administered SB 222200 had an attenuated stereotypic response to cocaine compared to vehicle treated mice. Mice were also injected once daily with either vehicle or SB 222200 (5 mg/kg, s.c.) for 5 days, and after a 7-day drug-free period they were challenged with either saline, cocaine or the dopamine D1 receptor agonist SKF 82958 (0.125 or 0.25 mg/kg, i.p.). Mice injected with SB 222200 had significantly enhanced hyperactivity when challenged with cocaine or a low dose of SKF 82958 (0.125 mg/kg, i.p.) compared to control mice. Brains of mice administered vehicle or SB 222200 for 5 days were harvested after a 7-day drug-free period for dopamine D1 receptor quantification by radioligand binding. [^{3}H] SCH 23390 homogenate binding studies showed a 19.7% increase in dopamine D1 receptor density in the striatum of SB 222200 treated mice. These data suggest that repeated blockade of NK-3 receptors enhances subsequent dopamine-mediated behaviors possibly resulting from dopamine D1 receptor up-regulation in the striatum.

1. Introduction

The mammalian tachykinin family of neuropeptides that includes substance P, neurokinin A, neurokinin B, neuropeptide K and neuropeptide gamma exert their biological actions through G-protein coupled receptors termed neurokinin-1, neurokinin-2, and neurokinin-3 (NK-3) (Maggi, 1995; Massi et al., 2000). Activation of NK-3 receptors primarily causes phosphoinositol 4, 5 biphosphate (PIP_2) breakdown into 1,4,5 inositol triphosphate (IP_3) and diacylglycerol through phospholipase C activation (Khawaja and Rogers, 1996; Maggi, 1995), eventually leading to Ca^{2+} mobilization and induction of Ca^{2+}-dependent downstream signaling pathways.

NK-3 receptors are found peripherally on nerve endings of primary afferent neurons innervating the respiratory, gastrointestinal and urinary tracts (Patacchini and Maggi, 2001), however they are also differentially expressed in the central nervous system. NK-3 receptors localized in the substantia nigra, ventral tegmental area (VTA) and prefrontal cortex (Dam et al., 1990) are thought to regulate dopaminergic neurotransmission and locomotive behaviors (Elliott et al., 1991; Overton et al., 1992). Tyrosine hydroxylase-containing neurons in the substantia nigra and VTA have been shown to express NK-3 receptors (Chen et al., 1998). Activation of NK-3 receptors in the substantia nigra and VTA stimulates dopaminergic neuronal activity (Keegan et al., 1992; Overton et al., 1992), and increases dopamine release and metabolism in the nucleus accumbens, striatum, and prefrontal cortex (Bannon et al., 1995; Humpel et al., 1991; Marco et al., 1998). In addition, NK-3 receptor activation elicits behaviors such as locomotion, rearing, sniffing and wet dog shakes in rats (Deschamps and Couture, 2005; Elliott et al., 1991; Jocham et al., 2007; Stoessl et al., 1991), which are diminished by administration of the dopamine D1 receptor antagonist SCH 23390 (Deschamps and Couture, 2005). In addition, NK-3 receptor activation potentiates locomotion induced by cocaine in rodents (Jocham et al., 2007) and also potentiates cocaine-induced decreases in exploratory activity, aerial scanning, and terrestrial glancing in non-human primates (de Souza Silva, 2006a). Conversely, dopamine D1 receptor-mediated and cocaine-induced behaviors are attenuated after administration of NK-3

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receptor antagonists in rats (Bishop and Walker, 2004; Jocham et al., 2006) and in cocaine responsive non-human primates (de Souza Silva, 2006b). These findings demonstrate that NK-3 receptors can modulate dopaminergic function and behavior.

To date, the majority of studies on NK-3 receptors and dopamine have examined modulation of dopaminergic function after acute administration of NK-3 receptor agonists and antagonists. However, effects of prior repeated blockade of NK-3 receptors on dopaminergic function have not been investigated. Since acute blockade of NK-3 receptors diminishes dopamine-mediated function and behavior, we hypothesized that repeated NK-3 receptor blockade may result in dopamine receptor super-sensitivity and enhancement of dopamine-mediated behaviors. Supporting evidence shows that dopamine-mediated behaviors are enhanced after previous chronic administration of dopamine receptor antagonists, and the change in dopamine-mediated behaviors results from up-regulation of dopamine receptors in the striatum (Hess et al., 1986, 1988). In particular, we propose that dopamine D1 receptor mediated behaviors and striatal dopamine D1 receptors may be altered after repeated NK-3 receptor blockade since previous studies have demonstrated that NK-3 receptor antagonists can attenuate dopamine D1 receptor-stimulated locomotion (Bishop and Walker, 2004). Therefore, the objectives of the present study were to investigate the effects of acute and repeated NK-3 receptor blockade on subsequent behavioral responses to cocaine, an indirect dopamine receptor agonist, and the selective dopamine D1 receptor agonist SKF 82958, and to examine changes in striatal dopamine D1 receptors that possibly underlie the change in behavioral response.

2. Materials and methods

2.1. Animals

Adult male CD-1 mice (Charles River Laboratories, Raleigh, NC, USA) were group-housed (4–6 per cage) in a temperature and humidity controlled environment on a 12-h light–dark cycle (lights on at 7AM) with *ad libitum* access to food and water. Animals were handled daily prior to the beginning of experiments. All experiments were conducted in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory animals and with approval from Temple University School of Medicine Institutional Animal Care and Use Committee.

2.2. Drugs and chemicals

Cocaine hydrochloride was generously provided by the National Institute on Drug Abuse and dissolved in a sterile 0.9% saline solution. The dopamine D1 receptor agonist 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF 82958) was obtained from Sigma–Aldrich (St. Louis, MO, USA) and dissolved in sterile saline. The NK-3 receptor antagonist (*S*)-3-methyl-2-phenyl-N-(1-phenylpropyl)-4-quinolinecarboxamide (SB 222200) was also obtained from Sigma–Aldrich and dissolved in a vehicle composed of 60% polyethylene glycol (PEG-200) and 40% distilled water. [^{3}H] SCH 23390 (84 Ci/mmol) was obtained from Perkin Elmer (Waltham, MA, USA). Fluphenazine and mianserin were both obtained from Sigma–Aldrich. Cocaine and SKF 82958 were injected intraperitoneally at volumes of 3 ml/kg, and SB 222200 was injected subcutaneously at a volume of 2 ml/kg.

2.3. Drug administration and behavioral assessment

The 4-quinolinecarboxamide based compound SB 222200 was used in our study to antagonize NK-3 receptors. It has been shown to be a centrally active compound with 57-fold selectivity for NK-3 versus NK-2 receptors and 100,000-fold selectivity for NK-3 versus NK-1 receptors (Sarau et al., 2000). SB 222200 has also been shown to be efficacious in inhibiting NK-3 receptor mediated Ca^{2+} mobilization and behavioral responses in a concentration-dependent manner in mice (Sarau et al., 2001, 2000). The doses of SB 222200 and pretreatment time used were chosen based on the *in vivo* pharmacological properties of SB 222200 that have been previously reported (Sarau et al., 2000).

Adult male CD-1 mice were placed into activity monitors for 1 h and injected with either vehicle or the NK-3 receptor antagonist SB 222200 (2.5, 5 mg/kg, s.c.). Thirty minutes later they were injected with cocaine (20 mg/kg, i.p.), and behavioral responses were monitored for 60 min. Behavioral activity was measured using the Digiscan DMicro System (Accusan, Columbus, OH, USA) that consisted of clean clear 20 × 20 × 42 cm plastic cages lined with horizontal photo-beams and detectors connected to an output computer. Activity was recorded as number of photo-beam breaks as the animal moved about the cage. Ambulatory activity and stereotypic activity data were obtained from recorded number of consecutive and repetitive beam breaks, respectively.

To examine effects of repeated NK-3 receptor blockade on behavioral response to cocaine, mice were injected once daily with either vehicle or SB 222200 (5 mg/kg, s.c.) for 5 days (Days 1–5), followed by a 7-day drug-free period. On day 13, they were challenged with either saline, cocaine (20 mg/kg, i.p.), or SKF 82958 (0.125, 0.25 mg/kg, i.p.), and behavioral activity was measured. A separate group of mice were euthanized on day 13, and their brains were harvested for measurement of dopamine D1 receptor density by [^{3}H] SCH 23390 homogenate receptor binding as described below.

2.4. Dopamine D1 receptor homogenate binding

Striata were rapidly dissected on ice, pooled from 4 mice, and homogenized in 50 mM Tris HCl (pH 7.4 at 4 °C) with a polytron. Homogenates were centrifuged at 30,000 × g for 15 min at 4 °C, pellets were resuspended in fresh cold Tris HCl, and were centrifuged again. The pellets were resuspended and incubated in a 37 °C shaking water bath for 30 min. The homogenates were centrifuged, and resuspended in 50 mM Tris HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ at pH 7.4 at room temperature for the binding assays. Striatal membranes were incubated at room temperature for 45 min with [^{3}H] SCH 23390 (0.1–8 nM) and 1 μM mianserin to block binding to 5-HT receptors. Non-specific binding was determined from membranes incubated with [^{3}H] SCH 23390 and mianserin in the presence of 10 μM fluphenazine. After incubation, membranes were harvested onto Whatman GF/B filter paper with cold Tris salt buffer using a Brandel cell harvester. Filter papers were transferred into vials filled with scintillation fluid (Cytoscint, Fisher Scientific) and radioactivity was counted by liquid scintillation. Protein concentrations of striatal homogenates were determined by the Lowry protocol (Lowry et al., 1951).

2.5. Data analysis

Acute behavioral data were analyzed by either an unpaired *t*-test or one-way ANOVA with Bonferroni post hoc tests using GraphPad Prism. Data from studies on behavioral responses to cocaine and the dopamine D1 receptor agonist SKF 82958 after repeated SB 222200 pretreatment were analyzed by two-way ANOVA with factors of Pretreatment (vehicle, SB 222200) and Challenge (saline, cocaine, SKF 82958), and further analyses were conducted using Bonferroni post hoc tests. Data from the [^{3}H] SCH 23390 receptor binding studies were analyzed by Scatchard analysis using GraphPad Prism, followed by an unpaired *t*-test. Statistical significance was determined at the alpha level of 0.05.

3. Results

3.1. Effect of acute administration of the NK-3 antagonist SB 222200 on behavioral responses to cocaine

Ambulatory and stereotypic responses to cocaine in mice pretreated acutely with either vehicle or SB 222200 were measured. One-way ANOVA of ambulatory activity revealed a significant difference between the treatment groups ($F(3,50) = 11.14$, $p < 0.0001$, Fig. 1a). Bonferroni post hoc comparisons showed that cocaine significantly increased ambulatory activity in vehicle pretreated animals ($p < 0.001$, vehicle-saline vs. vehicle-cocaine groups). There was no effect of SB 222200 on ambulatory activity induced by cocaine ($p > 0.05$, vehicle-cocaine vs. SB-cocaine groups). Statistical analysis of stereotypic activity revealed significantly different behavioral responses between the treatment groups ($F(3,50) = 13.13$, $p < 0.0001$, Fig. 2b). Post hoc comparisons showed a significant stereotypic response to cocaine in vehicle pretreated mice ($p < 0.001$, vehicle-saline vs. vehicle-cocaine groups). Stereotypic activity induced by cocaine was dose-dependently attenuated by SB 222200 with significance at the 5 mg/kg dose ($p < 0.05$, vehicle-cocaine vs. 5SB-cocaine groups). SB 222200 pretreatment by itself had no significant effects on either ambulatory or stereotypic activity ($p > 0.05$, vehicle-saline vs. 5SB-saline groups).

3.2. Effect of prior administration of SB 222200 on subsequent behavioral hyperactivity induced by cocaine

Mice were injected once daily with either vehicle or SB 222200 for five days. After a 7-day drug-free period they were challenged

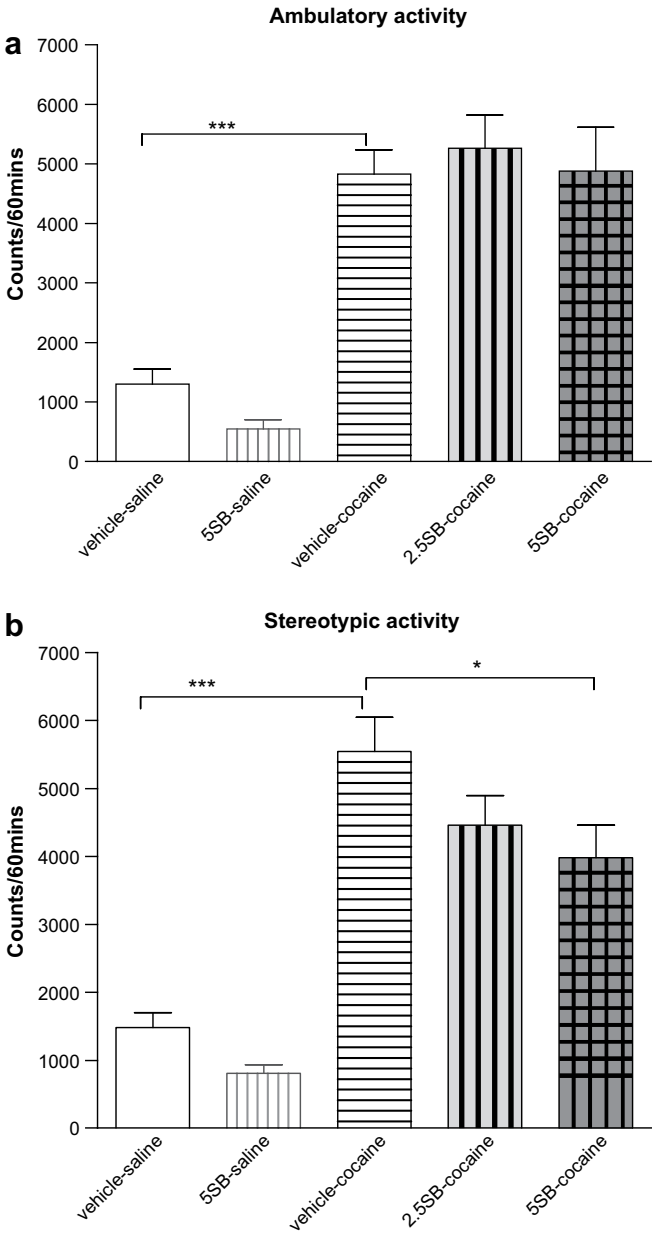

Fig. 1. Effect of acute NK-3 receptor blockade on cocaine-induced ambulatory (a) and stereotypic (b) activity. Adult male CD-1 mice were injected with either vehicle or the NK-3 receptor antagonist SB 222200 (2.5, 5 mg/kg s.c.) 30 min prior to either saline or cocaine (20 mg/kg i.p.). Within vehicle pre-treated groups, there was a significant increase in both ambulatory and stereotypic activity following cocaine. Pretreatment with SB 222200 significantly attenuated stereotypic activity in response to cocaine. Data are presented as mean ± SEM; $N = 6$–19 mice/group ($^{*}p < 0.05$, $^{***}p < 0.001$).

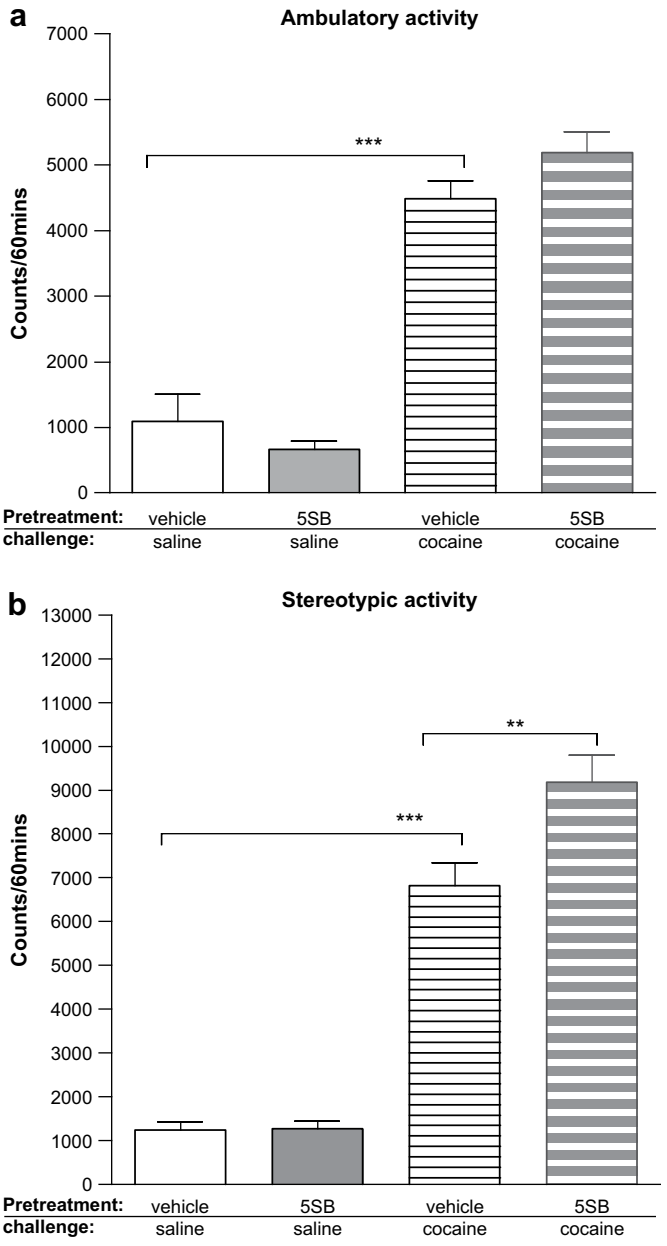

Fig. 2. Effect of prior NK-3 receptor blockade on ambulatory (a) and stereotypic (b) activity of mice after a cocaine challenge. Adult male CD-1 mice were injected once daily with either vehicle or the NK-3 receptor antagonist SB 222200 (5 mg/kg, s.c.) for five days, and on day 13 challenged with either saline or cocaine (20 mg/kg i.p.). Within vehicle pre-treated groups, cocaine increased both ambulatory and stereotypic activity. SB 222200 pretreatment significantly enhanced the behavioral response to cocaine in stereotypic activity (b) compared to vehicle. Data are presented as mean ± SEM; $N = 6$-12 mice/group ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

with either saline or cocaine, and behavioral activity was measured. Statistical analysis of ambulatory activity (Fig. 2a) showed a significant main effect of Challenge ($F(1,29) = 142.6$, $p < 0.0001$), but there was no effect of Pretreatment ($F(1,29) = 0.49$, $p > 0.05$) nor significant Pretreatment × Challenge interaction ($F(1,29) = 2.23$, $p > 0.05$). Cocaine significantly increased ambulatory activity (vehicle-saline vs. vehicle-cocaine groups) however pretreatment with SB 222200 for 5 days had no significant effect on ambulatory response to cocaine (vehicle-cocaine vs. 5SB-cocaine groups). Statistical analysis of stereotypic activity (Fig. 2b) revealed a significant main effect of Challenge ($F(1,29) = 155.7$, $p < 0.0001$) and Pretreatment ($F(1,29) = 4.88$, $p < 0.05$,) as well as a significant Pretreatment × Challenge interaction ($F(1,29) = 4.71$, $p < 0.05$). Bonferroni post hoc tests showed that cocaine induced a significant increase in stereotypic activity ($p < 0.001$, vehicle-saline vs. vehicle-cocaine groups). Furthermore, prior repeated administration of SB 222200 resulted in a significantly higher stereotypic response to cocaine as compared to the response after vehicle pretreatment ($p < 0.01$, vehicle-cocaine vs. SB-cocaine groups). These data show that cocaine-induced stereotypic behavior was enhanced after prior administration of the NK-3 receptor antagonist SB 222200. Repeated SB 222200 administration did not significantly alter either basal ambulatory or stereotypic activity ($p > 0.05$, vehicle-saline vs. SB-saline groups).

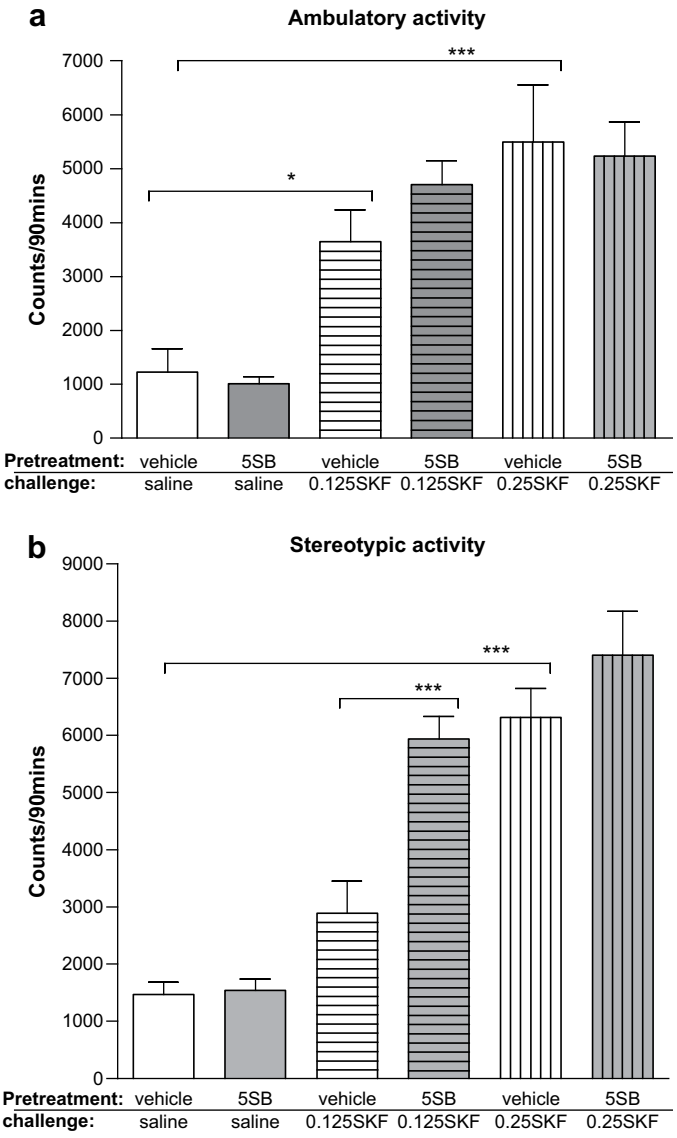

Fig. 3. Effect of prior NK-3 receptor blockade on ambulatory (a) and stereotypic (b) activity of mice after a challenge with the dopamine D1 receptor agonist SKF 82958. Adult male CD-1 mice were injected once daily with either vehicle or the NK-3 receptor antagonist SB 222200 (5 mg/kg, s.c.) for five days, and on day 13 challenged with either saline or SKF 82958 (0.125 or 0.25 mg/kg, i.p.). Vehicle pre-treated animals had a significant behavioral response to SKF 82958 in ambulatory activity (a) and in stereotypic activity (b) at the 0.25 mg/kg dose. Pretreatment with SB 222200 significantly enhanced stereotypic activity following a challenge injection of 0.125 mg/kg SKF 82958. Data are presented as mean ± SEM; $N = 5$–6 mice/group ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

3.3. Effect of prior administration of the NK-3 antagonist SB 222200 on dopamine D1 receptor-mediated behavioral activity

In order to study involvement of dopamine D1 receptors in the enhanced cocaine behavioral response after repeated NK-3 blockade, we examined changes in SKF 82958-mediated hyperactivity after repeated SB 222200 administration. Similar to the behavioral studies with cocaine, vehicle or SB 222200 was administered once daily for 5 days. After a 7-day drug-free period, animals were challenged with either saline or the dopamine D1 receptor agonist SKF 82958, and behavioral responses were measured. Analysis of ambulatory activity (Fig. 3a) showed a significant main effect of Challenge ($F(2,28) = 15.63$, $p < 0.0001$) but no effect of Pretreatment ($F(1,28) = 0.093$, $p > 0.05$), nor a Pretreatment × Challenge interaction ($F(2,28) = 0.46$, $p > 0.05$). Both doses of SKF 82958 induced a significant increase in ambulatory activity, however ambulatory activity was not altered by SB 222200. Statistical analysis of stereotypic activity (Fig. 3b) revealed a significant main effect of Challenge ($F(2,28) = 67.43$, $p < 0.0001$) and Pretreatment ($F(1,28) = 13.74$, $p < 0.001$) as well as a significant Pretreatment × Challenge interaction ($F(2,28) = 5.41$, $p < 0.01$). Bonferroni post hoc tests showed that 0.25 mg/kg SKF 82598 induced a significant increase in stereotypic activity in vehicle pretreated animals ($p < 0.001$, vehicle-saline vs. vehicle-0.25SKF groups), although the lower dose of SKF 82958 (0.125 mg/kg) did not significantly alter stereotypic activity ($p > 0.05$, vehicle-saline vs. vehicle-0.125SKF groups). Stereotypic activity following 0.125 mg/kg SKF 82958 was significantly higher in mice pretreatment with SB 222200 compared to those pretreated with vehicle ($p < 0.001$, vehicle-0.125SKF vs. SB-0.125SKF groups). These data demonstrate that prior administration of the NK-3 receptor antagonist SB 222200 enhanced stereotypic behavior to a sub-effective dose of the dopamine D1 receptor agonist SKF 82958. Repeated SB 222200 administration did not significantly alter either basal ambulatory or stereotypic activity ($p > 0.05$, vehicle-saline vs. SB-saline groups).

3.4. Dopamine D1 receptor up-regulation in the striatum of mice pretreated with the NK-3 receptor antagonist SB 222200

Since both behavioral responses to cocaine and SKF 82958 were enhanced following SB 222200 administration, the effect of SB 222200 on dopamine D1 receptor density was assessed. Mice were administered either vehicle or SB 222200 for 5 days and left drug-free for 7 days. On day 13, the striatum of animals were harvested to study changes in dopamine D1 receptor density by [^{3}H] SCH 23390 homogenate binding. Results from Scatchard analyses showed a significant increase in B_{max} of striatal membranes from SB 222200 injected mice as compared to vehicle injected mice (unpaired t-test; $t(6) = 2.537$, $p < 0.05$). B_{max} values of dopamine D1 receptor binding of striatal membranes from the SB treatment group were 19.7% higher than controls (Table 1, Fig. 4) indicating an increase in dopamine D1 receptor density in the striatum. There was no significant change in K_D values after SB 222200 administration (Table 1).

4. Discussion

Modulation of NK-3 receptor activity has been shown to alter dopamine-mediated behaviors. Previous studies have reported that administration of NK-3 receptor agonists elicits dopamine-mediated behaviors which include hyper-locomotion and stereotypy (Deschamps and Couture, 2005; Elliott et al., 1991; Stoessl et al.,

Table 1
B_{max} and K_D values from Scatchard analyses of [^{3}H] SCH 23390 homogenate binding assays. Adult male CD-1 mice were injected once daily with either vehicle or the NK-3 receptor antagonist SB 222200 (5 mg/kg, s.c.) for five days. After a 7-day drug-free period, dopamine D1 receptor density in the striatum was measured using [^{3}H] SCH 23390. Scatchard analyses showed an up-regulation of dopamine D1 receptors in the striatum of animals administered SB 222200 compared to animals injected with vehicle. Data are presented as mean ± SD; $N = 4$ separate assays ($^*p < 0.05$ vehicle vs. SB 222200).

	B_{max}	K_D
	fmol/mg protein	nM
Vehicle	742.5 ± 48.6	2.6 ± 0.3
SB 222200	888.5 ± 104.6*	2.3 ± 0.3
% change	+19.7	

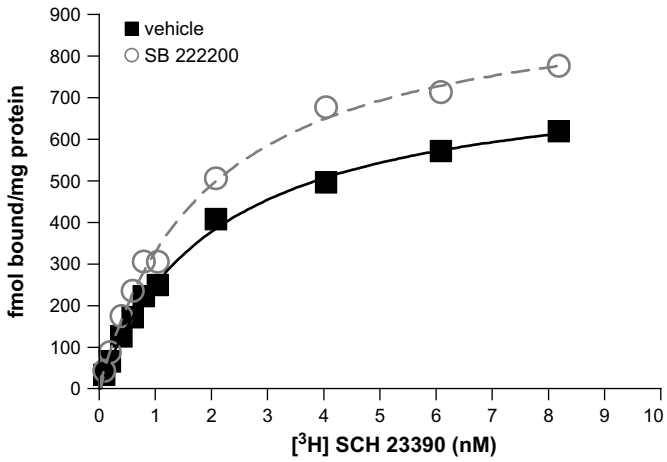

Fig. 4. [^{3}H] SCH 23390 binding to striatal membranes from mice injected with vehicle and SB 222200. Adult male CD-1 mice were injected once daily with either vehicle or the NK-3 receptor antagonist SB 222200 (5 mg/kg, s.c.) for five days, and on day 13 dopamine D1 receptor densities in striatal membranes were measured using [^{3}H] SCH 23390. Dopamine D1 receptor density was higher in striatal membranes from animals treated with SB 222200 compared to membranes from vehicle treated animals. A representative saturation binding curve is shown.

1991). The NK-3 receptor antagonist SR 142801 attenuates locomotion and stereotypic activity induced by the dopamine D1 receptor agonist SKF 82958 (Bishop and Walker, 2004) or cocaine in rats (Jocham et al., 2006) and non-human primates (de Souza Silva, 2006b), however by itself has no effect on basal dopamine levels (Marco et al., 1998) or basal behavioral activity (Bishop and Walker, 2004; de Souza Silva, 2006b; Jocham et al., 2006). In support of these findings, our study shows that acute blockade of NK-3 receptors by SB 222200 attenuated behavioral hyperactivity induced by cocaine, particularly stereotypic activity. In addition, acute administration of SB 222200 did not significantly alter basal activity.

One potential mechanism by which NK-3 receptors could modulate dopamine-mediated behaviors is through its effects on dopaminergic neuronal activity. Previous studies have shown the NK-3 receptor agonist senktide when administered into the substantia nigra and VTA of anesthetized rats (Overton et al., 1992) or applied locally on midbrain slices (Keegan et al., 1992) increases the firing rate of dopaminergic neurons. Further, activation of NK-3 receptors in the substantia nigra and VTA increases dopamine release and DOPAC tissue content in the striatum, nucleus accumbens and prefrontal cortex (Bannon et al., 1995; Humpel et al., 1991; Marco et al., 1998). In the present study, acute administration of the NK-3 receptor antagonist SB 222200 attenuated cocaine-induced hyperactivity. Since NK-3 receptors can modulate dopaminergic neuronal activity, it is likely that this mechanism is involved in the change in behavior.

Another mechanism by which NK-3 receptors could acutely regulate dopaminergic behaviors is by modulating the activity of striatal cholinergic interneurons. Cholinergic interneurons in the striatum have been shown to alter the excitability of GABAergic medium spiny neurons and thereby facilitate neurotransmission from the cortex or thalamus to regulate dopamine-mediated behaviors (Kawaguchi et al., 1995; Preston et al., 2000; Saka et al., 2002). Application of NK-3 receptor agonists causes a Ca^{2+}-dependent depolarization of striatal cholinergic interneurons (Preston et al., 2000) and a phospholipase C and protein kinase C-dependent release of acetylcholine in striatal slices (Arenas et al., 1991; Preston et al., 2000).

Long-term effects of prior NK-3 receptor blockade on dopamine-mediated hyperactivity have not been reported to date. Our findings demonstrate that prior repeated NK-3 receptor blockade by SB 222200 enhanced stereotypic hyperactivity induced by a cocaine challenge. Since cocaine inhibits the re-uptake of the dopamine, norepinephrine and serotonin, we also investigated effects of a selective dopamine D1 receptor agonist SKF 82958. Behavioral hyperactivity induced by SKF 82958 was enhanced after prior repeated NK-3 receptor blockade similar to our findings with cocaine. The observed changes in behavioral hyperactivity were accompanied by dopamine D1 receptor up-regulation in the striatum after prior NK-3 receptor blockade suggesting a possible mechanism for the augmented behavioral response to cocaine and SKF 82958. Collectively, these findings suggest that in addition to acutely modulating dopamine, NK-3 receptor blockade can produce long-term changes in dopamine-mediated behaviors and receptor expression.

The subsequent behavioral supersensitivity of dopamine receptors caused by repeated administration of NK-3 receptor antagonist SB 222200 was shown in the present study to be associated with increased density of dopamine D1 receptors in the striatum. We propose that the change in behaviors may be an outcome of prolonged depression of dopamine transmission in the striatum. Previous studies have shown that 6-OHDA lesions of dopaminergic terminals results in enhancement of dopamine-mediated behaviors (Breese et al., 1987). Likewise, chronic administration of dopamine receptor antagonists results in dopamine receptor supersensitivity, and subsequent enhancement of dopamine D1 and D2 receptor-mediated behaviors which are accompanied by dopamine receptor up-regulation (Hess et al., 1986, 1988). In addition, chronic administration of dopamine receptor antagonists enhances cocaine-mediated hyperactivity (Mattingly et al., 1996). Since acute blockade of NK-3 receptors attenuates dopaminergic transmission, prolonged blockade of NK-3 receptors might eventually lead to dopamine receptor supersensitivity. In the present study, the enhancement of subsequent behavioral responses to both cocaine and the dopamine D1 receptor agonist SKF 82958 and the increase in dopamine D1 receptor density in the striatum is suggestive of dopamine receptor supersensitivity after repeated NK-3 receptor blockade. Our behavioral findings agree with a recent report by Nordquist and colleagues that demonstrate slightly enhanced behavioral responses to amphetamine in mice with genetic deletion of the NK-3 receptor (Nordquist et al., 2008). However, unlike Nordquist and colleagues who show small decreases in striatal dopamine D1 receptors in NK-3 receptor null mice, we found increased dopamine D1 receptor binding in the striatum of mice 7 days after repeated NK-3 receptor blockade. Collectively, these findings point to compensatory changes in striatal dopamine D1 receptors and psychostimulant-induced behaviors following manipulation of NK-3 receptors. Furthermore, our study demonstrates long-term changes in dopamine-mediated behaviors and dopamine receptor expression that is possibly an outcome of prolonged depression of dopaminergic transmission.

Investigations of NK-3 receptor function in the central nervous systems are currently being conducted in various animal models that include mice, rats, gerbils, guinea pigs and non-human primates. There is evidence of differences in NK-3 receptor structure, expression in the central nervous system, and ligand selectivity among these species and also in humans (Buell et al., 1992; Langlois et al., 2001; Maggi, 1995; Mileusnic et al., 1999), which has presented some obstacles in the study of mammalian NK-3 receptors. For instance, in comparison of the rat, gerbil and guinea pig, the rat and gerbil has NK-3 receptor binding in the substantia nigra par compacta and the ventral tegmental area, and only the rat has NK-3 receptors in the anterior caudate putamen (Langlois et al., 2001). Species differences in the pharmacological characteristics of NK-3 receptor antagonists further complicate investigations in

these animal models. For instance, the NK-3 receptor antagonist SR 1421801 has the highest affinity for the guinea pig NK-3 receptor with a Ki value of 0.11 nM as compared to the rat (15 nM), gerbil (0.42 nM) and human (0.21 nM) NK-3 receptors (Emonds-Alt et al., 1995). The NK-3 receptor antagonist used in this study, SB 222200, also shows some species differences in binding with a Ki value of 4.4 nM for the human NK-3 receptor, 3 nM for the guinea pig, 88 nM for the rat and 174 nM for the mouse (Sarau et al., 2000). In addition, studies have shown there are differences in selectivity of the endogenous ligands NK-B and substance P for NK-3 receptors. The selectivity of NK-B over substance P is greater in the rat and mouse (Sarau et al., 2001; Shigemoto et al., 1990) as compared to the human NK-3 receptor that is less selective (Buell et al., 1992; Sarau et al., 2001). These species differences present some obstacles in the study of NK-3 receptors, but despite the presented limitations, our findings offer insight into the function of NK-3 receptors and possible long-term adaptations in dopaminergic behaviors as a result of repeated NK-3 receptor blockade.

NK-3 receptors are being studied as potential therapeutic targets for treatment of various human pathologies. In the periphery, NK-3 receptors can be found mainly on nerve endings of c-fibers of primary afferent neurons innervating the respiratory, gastrointestinal and urinary tracts (Patacchini and Maggi, 2001). In these areas, NK-3 receptors play a role in pathological inflammatory processes implicated in diseases such as asthma, inflammatory bowel syndrome and cystitis (Canning, 2006; Patacchini and Maggi, 2001). In the central nervous system, their role in modulating dopamine transmission makes them potential targets in treatment of neuropsychiatric illnesses including schizophrenia and various affective disorders, and also Parkinson's disease (Panocka et al., 2001; Ribeiro and De Lima, 1998; Spooren et al., 2005). Findings from the present study lead us to propose further inquiry into possible consequences of chronic use of agents that function as NK-3 receptor antagonists. Their long-term use may cause dopamine receptor supersensitivity, similar to what is observed with use of classical antipsychotics that can cause tardive syndromes.

In summary, our findings show that acute blockade attenuated but repeated blockade of NK-3 receptors enhanced subsequent dopamine-mediated behaviors, and concurrently up-regulated dopamine D1 receptors in the striatum. These findings point to a potential role of NK-3 receptors in mediating acute and long-term changes in dopaminergic transmission and indicate that there is a functional interaction between NK-3 receptors and dopamine neurotransmission in the striatum.

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