

## Altered neurotensin mRNA expression in mice lacking the dopamine transporter

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## ABSTRACT

Psychostimulants and antipsychotic drugs increase mRNA expression of the neuropeptide neurotensin (NT) in the striatum and nucleus accumbens. In the present study, we used mice lacking the dopamine transporter (DAT) to investigate the consequences of a chronic hyperdopaminergic state on NT gene expression. NT mRNA expression was examined under basal conditions and after administration of haloperidol or amphetamine using in situ hybridization with a digoxigenin-labeled NT cRNA probe. DAT<sup>-/-</sup> mice exhibited a striking increase in the number of NT mRNA-expressing perikarya in the substantia nigra and ventral tegmental area, as well as a less pronounced increase in the lateral septum compared to wild-type littermates. No changes were detected in other regions expressing NT mRNA. Acute administration of haloperidol (1 mg/kg) induced a significant increase in the number of NT mRNA-expressing neurons in the dorsomedial and dorsolateral striatum of wild-type mice but failed to stimulate NT gene expression in DAT mutants. In contrast, a higher dose of haloperidol (5 mg/kg) stimulated striatal NT mRNA expression both in DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice. Amphetamine (10 mg/kg) increased the number of hybridized neurons in the nucleus accumbens shell and fundus striati of wild-type and DAT<sup>-/-</sup> mice, indicating that the drug acted through a target other than DAT, such as the serotonin or the norepinephrine transporters. The up-regulation of NT mRNA observed in DAT<sup>-/-</sup> mice may represent an adaptive mechanism in response to constitutive hyperdopaminergia. These results illustrate the profound alterations in the NT system induced by chronic stimulation of DA receptors and underscore the potential clinical relevance of NT/DA interactions in schizophrenia and drug abuse.

**Key words:** amphetamine, haloperidol, in situ hybridization, knock-out mice, striatum, accumbens

**List of abbreviations**

accumbens nucleus, shell	AcbSh
anterior commissure	ac
dopamine	DA
dopamine transporter	DAT
dorsolateral striatum	DL
dorsomedial striatum	DM
fundus striati	FStr
lateral septum	LS
lateral ventricle	LV
messenger ribonucleic acid	mRNA
neurotensin	NT
norepinephrine transporter	NET
phosphate-buffered saline	PBS
reverse transcriptase polymerase chain reaction	RT-PCR
serotonin transporter	SERT
subiculum	S
substantia nigra	SN
substantia nigra, compact part	SNc
olfactory tubercle	Tu
ventral tegmental area	VTA
ventrolateral striatum	VL

**Running title:** Neurotensin mRNA expression in DAT<sup>-/-</sup> mice

Dopamine (DA) is an important neuromodulator of numerous physiological functions, including locomotion, cognition, motivation, food intake, and endocrine regulation (for review, see Grace et al., 1998). Abnormal dopaminergic transmission has been implicated in several neuropsychiatric disorders, including schizophrenia and drug addiction (Carlsson, 1988; Koob and Nestler, 1997). The dopamine transporter (DAT) is responsible for the rapid removal of DA from the synaptic cleft and therefore regulates the availability of synaptic DA and its subsequent interaction with pre- and postsynaptic receptors (Giros and Caron, 1993). Mice in which the DAT gene has been inactivated by genetic manipulation (DAT<sup>-/-</sup>) display locomotor hyperactivity and a dramatic increase in extracellular DA levels in the brain (Giros et al., 1996). This phenotype is accompanied by numerous pre- and postsynaptic adaptive changes, including decreased D<sub>1</sub> and D<sub>2</sub> receptor mRNA levels and increased D<sub>3</sub> receptor mRNA expression in the striatum and nucleus accumbens (Giros et al., 1996; Fauchey et al., 2000a), loss of D<sub>2</sub> autoreceptor functions (Jones et al., 1999), and increased D<sub>1</sub> receptor sequestration in the cytoplasmic compartment (Dumartin et al., 2000). Furthermore, the expression of genes encoding neuropeptides preproenkephalin and dynorphin, which is under dopaminergic control, shows variations indicative of increased dopaminergic tone. Thus, preproenkephalin A mRNA expression, under the inhibitory influence of DA by means of D<sub>2</sub> receptors (Gerfen et al., 1990), is down-regulated in the caudate putamen and nucleus accumbens of DAT<sup>-/-</sup> mice (Giros et al., 1996; Fauchey et al., 2000b). In contrast, dynorphin mRNA expression, under the excitatory control of DA by means of D<sub>1</sub> receptors (Gerfen et al., 1990), is up-regulated in these mutant mice (Giros et al., 1996; Fauchey et al., 2000b).

To further investigate the consequences of long term hyperdopaminergic tone in DAT<sup>-/-</sup> mice, we studied the expression of the peptide neurotransmitter neurotensin (NT). NT mRNA expression is stimulated in the dorsal striatum and shell of the nucleus accumbens by administration of D<sub>2</sub> receptor antagonists like the antipsychotic haloperidol (Merchant and Dorsa, 1993), as well as by indirect dopaminergic agonists like the psychostimulants amphetamine and cocaine (Castel et al., 1994; Merchant et al., 1994; Betancur et al., 1997). These observations, together with numerous neurochemical, electrophysiological, anatomical, and behavioral studies indicating that NT modulates DA transmission in the nigrostriatal and mesolimbic pathways (for review, see Binder et al., 2001a), suggest that alterations in NT transmission may be involved in the mechanism of action of neuroleptics and psychostimulant drugs. Traditionally, NT has been proposed to act as an endogenous neuroleptic (Nemeroff, 1980), and has been implicated in the pathophysiology of schizophrenia (Binder et al., 2001b), although more recent data suggest that it could also act as an endogenous psychostimulant (Bérod and Rostène, 2002).

In the present study we examined NT mRNA levels in DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice under basal conditions and after acute administration of haloperidol and amphetamine, using *in situ* hybridization with a digoxigenin-labeled NT cRNA probe.

## EXPERIMENTAL PROCEDURES

### Animals

Homozygous DAT<sup>-/-</sup> mice were obtained by homologous recombination (Giros *et al.*, 1996) and back-crossed for 12 generations on a C57BL/6 background. DAT<sup>-/-</sup> and DAT<sup>+/+</sup> mice were obtained from mating of DAT<sup>+/-</sup> mice and their genotypes were determined by Southern blot analysis as previously described (Giros *et al.*, 1996). Mice were housed four per cage under a 12-hour light-dark cycle, at 21±1 °C ambient temperature, and given chow and water *ad libitum*. Additional moisturized chow was provided every day to increase the survival rate of DAT<sup>-/-</sup> mice. The experiments were performed when mice were 10-12 weeks old. All experiments were conducted in accordance with standard ethical guidelines (European Communities Council Directive for the care and use of laboratory animals) and approved by the local ethical committee. All efforts were made to minimize the number of animals used and their suffering.

### Drug treatments

Mice (n=3-6 per group) received an acute *i.p.* injection of haloperidol 1 or 5 mg/kg (Sigma, St-Quentin Fallavier, France), d-amphetamine sulfate 10 or 30 mg/kg (Sigma), or saline, 6 h before sacrifice. This survival time was chosen on the basis of previously published studies showing maximal induction of NT mRNA 3-7 h after haloperidol (Merchant *et al.*, 1993) and 2-6 h after amphetamine (Castel *et al.*, 1994).

### Preparation of mouse NT riboprobe

Mice were killed by decapitation and the substantia nigra was dissected at 0–4°C, frozen in liquid nitrogen and stored at –80°C for subsequent RNA extraction. Total RNA was extracted using the acid guanidinium thiocyanate method (Chomczynski and Sacchi, 1987), treated with DNase, recovered by phenol-chloroform extraction and ethanol precipitation, and quantified by spectrophotometry (optical density at 260 nm).

A 456 base-pair fragment was amplified with specific mouse NT primers (5'-AGACATGCATGATGACGACC-3'; 5'-ATGCAAGACACATCACATCC-3') (GenBank accession number AF348489) by reverse transcriptase polymerase chain reaction (RT-PCR) using the

Access RT-PCR system (Promega, Charbonnière, France). This RT-PCR product was subcloned into a PRC2.1 vector (Invitrogen, Carlsbad, CA, USA) and transformed according to the manufacturer's instructions. Clones bearing the right NT probes were isolated and their orientation was determined by restriction analysis. Two riboprobes, having different orientations in the vector, were prepared and cut with *Bam*HI to obtain antisense and sense probes. The probes were labeled with the T7-labeling kit (Tebu, Le Perray-en-Yvelines, France) using digoxigenin-labeling mix (Roche Molecular Biochemicals, Mannheim, Germany).

### **In situ hybridization histochemistry**

Cryostat sections (10  $\mu$ m) were heated for 10 min at 60°C and fixed for 1 h with 3.7% formaldehyde in phosphate-buffered saline (PBS) at 4°C, rinsed twice in PBS and dehydrated through a graded series of ethanol concentrations (50%–70%). Sections were then incubated overnight in the presence of 10 ng of digoxigenin-labeled antisense NT riboprobe in 100  $\mu$ l of buffer containing 50% formamide at 60°C. Hybridized neurons were detected by exposing the sections overnight to anti-digoxigenin antibodies conjugated to alkaline phosphatase (Roche Molecular Biochemicals) at a dilution of 1:1,000, and revealed using a mixture containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate toluidinium (Roche Molecular Biochemicals). Sections were then rinsed twice in PBS, covered with a glass coverslip, and sealed with Aquamount (Roche Molecular Biochemicals).

Quantification of labeled cells was performed using ImageQuant software (Molecular Dynamics, Amersham Pharmacia Biotech). Brain structures were identified according to the atlas of Franklin and Paxinos (1997). The number of hybridized neurons was analyzed by an investigator blind to treatment and genotype at two striatal levels (1.1 and 0.5 mm anterior to bregma), and at the level of the mesencephalon (–3.08 mm posterior to bregma), shown in Fig. 1. The following regions were analyzed at the rostral level (1.1 mm anterior to bregma): nucleus accumbens shell, olfactory tubercle, and dorsolateral, dorsomedial and ventrolateral striatum. The lateral septum was analyzed at the middle level (0.5 anterior to bregma). At the caudal level, measurements were performed in the substantia nigra (SN), ventral tegmental area (VTA), and subiculum. The areas where the cell counting was performed were standardized across sections and animals using anatomical landmarks. The analysis was performed bilaterally on 4–6 brain sections per animal at each level.

## Statistical analysis

Significant differences in basal NT mRNA expression between DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice were determined with the Student's *t*-test. A two-factor analysis of variance (ANOVA) was used to analyze the effects of haloperidol and amphetamine; post-hoc individual comparisons were performed with Tukey test. Statistical significance was set at  $P \leq 0.05$ .

## RESULTS

### Basal NT mRNA expression in DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice

Basal expression of NT mRNA was markedly increased in the SN compacta and VTA of DAT<sup>-/-</sup> mice compared with DAT<sup>+/+</sup> mice (Table 1 and Fig. 2E and F). Whereas there were only few detectable NT mRNA-expressing neurons in the ventral mesencephalon of wild-type animals, the number of labeled cells was increased five-fold in the SN compacta and ten-fold in the VTA of DAT<sup>-/-</sup> mutant mice (Student's *t*-test,  $P < 0.001$  and  $P < 0.01$ , respectively). The lateral septum of DAT<sup>-/-</sup> mice also displayed a 35% increase in the number of NT mRNA-expressing cells ( $P < 0.05$ ; Fig. 2C and D). In contrast, the subiculum was intensely labeled in both DAT<sup>-/-</sup> and DAT<sup>+/+</sup> mice, and no differences were observed between the two genotypes in the number of hybridized neurons (Fig. 2G and H). Furthermore, no significant changes in the number of NT mRNA-containing neurons or the intensity of staining of individual hybridized cells were detected in the nucleus accumbens shell, olfactory tubercles (Fig. 2A and B), or striatum in DAT<sup>-/-</sup> mice compared with wild-type littermates. Surprisingly, NT mRNA was not detected in the CA1 region of the hippocampus in DAT<sup>-/-</sup> and DAT<sup>+/+</sup> mice (not shown), although this region is known to contain high levels of this transcript in rats (Alexander et al., 1989).

### Effect of acute haloperidol administration

As shown in Fig. 3 and Table 2, acute administration of haloperidol 1 mg/kg i.p. increased the number of NT mRNA-containing neurons in the dorsomedial, dorsolateral, and ventrolateral striatum of wild-type mice 6 h after injection. In contrast, this increase was not observed in DAT<sup>-/-</sup> mice. However, at a higher dose, 5 mg/kg, haloperidol produced a greater increase in the number of hybridized cells in DAT<sup>+/+</sup> mice, and stimulated NT expression in DAT<sup>-/-</sup> mice in the three striatal regions. The ANOVA revealed a treatment effect ( $F(2,23)=87.1$ ,  $P < 0.0001$ ), no genotype effect ( $F(1,23)=0.7$ ,  $P < 0.42$ ), and a genotype x treatment interaction ( $F(2,23)=13.0$ ,  $P < 0.001$ ). The results of the post-hoc tests are indicated in Table 2.

Although administration of the antipsychotic drug at the higher dose did not induce an increase in the number of NT mRNA-positive cells in the shell of the nucleus accumbens, it



enhanced the intensity of staining of NT mRNA-containing neurons in both DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice (not shown).

### Effect of acute amphetamine administration

Administration of amphetamine (10 mg/kg i.p.) produced a robust increase in the number of NT mRNA-expressing neurons in a discrete region of the ventrolateral striatum, the fundus striati (Fig. 4) in both DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice (vehicle:  $28.8 \pm 2.2$  and  $23.7 \pm 3.7$ , amphetamine:  $54.5 \pm 3.2$  and  $45.8 \pm 6.2$ ; ANOVA followed by Tukey test,  $P < 0.01$  and  $P < 0.05$  in DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice, respectively). Amphetamine also increased the number of NT mRNA-containing neurons in the shell of the nucleus accumbens in wild-type and DAT<sup>-/-</sup> mutant mice (not shown). Similar effects were observed when mice of either genotype were injected with a higher dose of the psychostimulant (30 mg/kg; not shown). In contrast, no changes in the number of NT mRNA-positive cells were detected in the other striatal territories analyzed at the rostral level (dorsolateral and dorsomedial striatum), in the lateral septum, and the olfactory tubercles, in DAT<sup>-/-</sup> or DAT<sup>+/+</sup> mice treated with amphetamine (10 or 30 mg/kg).

## DISCUSSION

In the present study we examined the adaptive changes in NT mRNA expression in mice lacking the DAT and their response to amphetamine and haloperidol. In particular, we wanted to investigate the consequences of the long term hyperdopaminergic tone characteristic of DAT<sup>-/-</sup> mice in the regulation of the neurotensinergic system.

We first compared basal NT mRNA expression in DAT<sup>-/-</sup> and wild-type mice and found that DAT mutant mice exhibited a marked increase in NT mRNA levels in the VTA and SN compacta, and a less pronounced increase in the lateral septum. Deletion of the DAT gene results in a five- to ten-fold increase in extracellular DA level in the striatum and nucleus accumbens (Jones et al., 1998; Spieleswoy et al., 2000a). Although this increase in DA is similar to the one elicited by the administration of psychostimulants in wild-type mice (Carboni et al., 2001), the regional pattern of induction of NT mRNA in DAT<sup>-/-</sup> mice differs from that produced by psychostimulants. Cocaine and amphetamine increase NT mRNA expression in the shell of the nucleus accumbens, the caudal dorsomedial striatum, and the fundus striati, in rats (Castel et al., 1994; Merchant et al., 1994; Betancur et al., 1997; Adams et al. 2001) as well as in mice (Betancur et al., 2001). Up-regulation of NT mRNA levels is not observed in the lateral septum of rats or mice treated acutely or chronically with cocaine (Betancur et al., 1997, 2001). Moreover, no evidence has been reported for regulation of NT transcripts in the



SN or VTA after psychostimulant administration, although NT immunoreactivity is increased in these regions after single or multiple cocaine or methamphetamine injections in rats (Letter et al., 1987; Hanson et al., 1989). Interestingly, Hanson et al. (1989) showed that nigral NT systems were much more sensitive to cocaine administration than the striatum and nucleus accumbens. This observation supports our findings in DAT<sup>-/-</sup> mice showing that chronic hyperdopaminergia resulted in a dramatic up-regulation of NT mRNA levels in the SN/VTA, whereas no changes were observed in the striatum or nucleus accumbens. Another observation that could shed light on our results is the study by Feldpausch et al. (1998) showing long-lasting desensitization of NT mRNA responses in the nucleus accumbens shell after repeated exposure to amphetamine. Thus, the lack of up-regulation of NT mRNA levels in the nucleus accumbens of DAT<sup>-/-</sup> mice might also reflect desensitization after prolonged exposure to elevated DA levels. It should be noted however, that other transcriptional events resulting from the absence of DAT could also be involved in the increase in NT mRNA expression in nigral and VTA neurons.

What could be the functional consequences of the up-regulation of NT mRNA expression in DAT<sup>-/-</sup> mice, and their adaptive role on the altered DA system of these mice? NT can exert opposite effects, depending on whether it is administered in the cell body region or in the projection areas of dopaminergic neurons. When injected directly into the nucleus accumbens, NT reduces DA outflow from synaptic terminals (Tanganelli et al., 1994) as well as locomotor activity induced by amphetamine (Ervin et al., 1981; Kalivas et al., 1984). Conversely, NT administered into the VTA increases the activity of mesolimbic DA neurons, leading to DA release in the nucleus accumbens (Kalivas and Duffy, 1990) and enhanced locomotion (Kalivas et al., 1983). According to these data, the up-regulation of NT mRNA levels in the SN/VTA of DAT<sup>-/-</sup> mice, probably leading to enhanced release of NT from the terminals of nigrostriatal and mesolimbic pathways in the striatum and nucleus accumbens, might be an attempt to counterbalance the effects of elevated levels of extracellular DA present in these mice.

To further characterize the adaptive changes of the NT system in DAT<sup>-/-</sup> mutant mice, we investigated the effects of an indirect DA agonist and a DA receptor antagonist on NT mRNA expression. Acute haloperidol (1 mg/kg) failed to up-regulate NT mRNA expression in the dorsal striatum of DAT<sup>-/-</sup> mice, although it increased it in wild-type mice and rats (Merchant and Dorsa 1993; Zahm et al., 1998; Betancur et al., 2001). However, a higher dose of haloperidol (5 mg/kg) induced a marked increase in the number of hybridized neurons in DAT<sup>-/-</sup> and DAT<sup>+/+</sup> mice. Similarly, higher doses of haloperidol are necessary to reduce locomotion in DAT<sup>-/-</sup> mice compared to DAT<sup>+/+</sup> mice (Spielewoy et al., 2000b). Previous

studies have shown that the effects of haloperidol on NT gene transcription involve blockade of D<sub>2</sub> receptors (Merchant et al., 1989; Augood et al., 1991). In DAT<sup>-/-</sup> mice, D<sub>2</sub> receptor mRNA levels are decreased by 36%-45% in the caudate putamen and nucleus accumbens (Fauchey et al., 2000a; Giros et al., 1996), and the function of the remaining receptors is decreased by approximately 90% (Jones et al., 1999). Thus, it is possible that decreased D<sub>2</sub> receptor number and function contribute to the diminished potency of haloperidol to up-regulate NT mRNA in mice lacking the DAT. Alternatively, higher doses of haloperidol may be required in order to displace the enhanced levels of endogenous synaptic DA found in DAT<sup>-/-</sup> mice (Giros et al., 1996).

In the rat, Merchant et al. (1993, 1994) described an upregulation of NT mRNA levels after haloperidol administration only in the dorsolateral striatum. In contrast, in the present study in mice, haloperidol enhanced NT gene transcription in the dorsomedial and dorsolateral striatum, and to a lesser extent, in the ventrolateral region. This is very likely an interspecies difference, since similar findings were reported recently in D<sub>3</sub> receptor-deficient mice and wild-type animals (Betancur et al., 2001).

Amphetamine induced comparable increases in NT mRNA levels in the shell of the nucleus accumbens and in the fundus striati of DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice. Previous studies have shown that the effects of amphetamine on NT mRNA expression involve the activation of D<sub>1</sub> receptors (Castel et al., 1993). DAT<sup>-/-</sup> mice exhibit a 34%-55% decrease in D<sub>1</sub> receptor mRNA levels in the caudate putamen and nucleus accumbens (Giros et al., 1996; Fauchey et al., 2000a), together with accumulation of the receptor in the cytoplasmic compartment and decreased delivery to the plasma membrane (Dumartin et al., 2000). Despite these profound compensatory changes, psychostimulant-induced NT mRNA expression was not disturbed in DAT<sup>-/-</sup> mice.

Classically, the effects of amphetamine have been considered to depend on the ability of the drug to block DAT, thereby increasing the extracellular concentration of DA (Giros and Caron, 1993; Koob and Nestler, 1997). The elevated NT mRNA expression induced by amphetamine in mice lacking DAT suggests that this effect is likely to be mediated by the blockade of the serotonin transporter (SERT) or the norepinephrine transporter (NET). Indeed, amphetamine blocks with similar affinity DAT, SERT and NET (Amara and Kuhar 1993). In agreement with our findings, recent studies have shown that cocaine, another drug thought to act by blocking DAT, is still able to induce self-administration and conditioned place preference in DAT<sup>-/-</sup> mice (Rocha et al., 1998; Sora et al., 1998). The rewarding effects of cocaine observed in DAT<sup>-/-</sup> mice have been suggested to involve an interaction with SERT (Rocha et al., 1998), and a recent study using combined DAT and SERT knockout mice

showed that cocaine place preference is absent in these mice (Sora et al., 2001). According to these findings, it is possible that in the absence of DAT, the action of amphetamine on NT mRNA expression involves blockade of SERT, and the suppression of both DAT and SERT would be necessary to abolish the response to amphetamine. In support of this hypothesis, SERT is highly expressed in the striatum and nucleus accumbens (Duncan et al., 1992). However, SERT blockade alone is unlikely to account for the amphetamine-induced increase in NT mRNA levels in the nucleus accumbens and fundus striati in DAT<sup>-/-</sup> mice because fenfluramine (5 mg/kg i.p.) and fluoxetine (10 mg/kg i.p.), two potent SERT inhibitors, failed to up-regulate NT transcripts in these two regions in DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice (data not shown).

Recent findings suggest that NET could be implicated in NT mRNA induction by amphetamine. Indeed, cocaine and amphetamine are still able to increase extracellular DA levels in the nucleus accumbens of DAT<sup>-/-</sup> mice by blocking DA reuptake mediated by NET (Carboni et al., 2001). In the absence of DAT, the specific NET blocker reboxetine increased DA levels in the nucleus accumbens, but had no effect in wild-type mice (Carboni et al., 2001). In fact, NET is able to transport DA with a higher affinity than DAT itself (Giros and Caron, 1993), and under normal circumstances is responsible for the majority of DA re-uptake in the frontal cortex, while playing a smaller contribution than DAT in the striatum and nucleus accumbens (Moron et al., 2002). Therefore, the effects of amphetamine on NT mRNA expression in DAT<sup>-/-</sup> mice could be mediated by increased extracellular DA levels secondary to blockade of NET.

In conclusion, the study of NT systems in mice lacking the DAT showed a strong up-regulation of NT mRNA expression in regions containing dopaminergic cell bodies and terminals, reflecting the chronically increased dopaminergic neurotransmission observed in these mice. The overexpression of NT mRNA in the SN/VTA in DAT<sup>-/-</sup> mice may represent a compensatory mechanism to counterbalance chronic and constitutive hyperdopaminergia. These results illustrate the profound alterations in the NT system induced by chronic stimulation of DA receptors and may have potential clinical relevance to psychiatric disorders associated with abnormal dopaminergic transmission, such as schizophrenia and drug addiction.

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Table 1. Basal NT mRNA expression in DAT+/+ and DAT–/– mice.

Brain regions	DAT+/+	DAT–/–
Nucleus accumbens shell	147.2 ± 17.3	164.6 ± 23.8
Olfactory tubercle	47.2 ± 9.9	63.5 ± 18.6
Lateral septum	105.4 ± 6.3	142.7 ± 11.3 *
Dorsomedial striatum	1.7 ± 0.5	2.4 ± 0.8
Dorsolateral striatum	1.8 ± 0.8	3.2 ± 0.9
Ventrolateral striatum	1.9 ± 0.9	2.1 ± 0.7
Substantia nigra, compact part	11.0 ± 0.7	61.0 ± 5.1 ***
Ventral tegmental area	5.0 ± 2.3	46.8 ± 7.4 **
Subiculum	94.3 ± 0.3	86.0 ± 3.3

Values represent the number of NT mRNA-expressing neurons (mean ± S.E.M.), n = 3-6 mice per group. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , Student's *t*-test.

Table 2. Effect of acute haloperidol administration on NT mRNA expression in the striatum of DAT+/+ and DAT–/– mice.

Treatment	Genotype	DM Striatum	DL Striatum	VL Striatum
Vehicle	DAT+/+ (n = 5)	1.7 ± 0.5	1.8 ± 0.8	1.9 ± 0.9
	DAT–/– (n = 6)	2.4 ± 0.8	3.2 ± 0.9	2.1 ± 0.7
Haloperidol 1 mg/kg	DAT+/+ (n = 3)	41.7 ± 6.4 *	28.4 ± 8.1 *	8.4 ± 2.8
	DAT–/– (n = 4)	4.2 ± 1.2	1.3 ± 0.3 <sup>+</sup>	0.1 ± 0.1
Haloperidol 5 mg/kg	DAT+/+ (n = 3)	65.2 ± 8.4 **	60.7 ± 10.7 **	46.4 ± 6.0 **
	DAT–/– (n = 3)	110.0 ± 26.7 ** <sup>+</sup>	111.4 ± 11.3 ** <sup>++</sup>	77.9 ± 18.3 ** <sup>+</sup>

Values represent the number of NT mRNA-expressing neurons (mean ± S.E.M.). DM, dorsomedial; DL, dorsolateral; VL, ventrolateral. \*  $P < 0.05$ , \*\*  $P < 0.01$ , vs. vehicle-treated mice of the same genotype; <sup>+</sup>  $P < 0.05$ , <sup>++</sup>  $P < 0.01$ , vs. DAT+/+ mice; ANOVA followed by Tukey test.

## FIGURE LEGENDS

**Fig. 1.** Schematic diagrams showing the regions where NT mRNA-expressing neurons were counted at three rostro-caudal levels of the mouse brain: bregma 1.1 mm (top), 0.5 mm (middle), and -3.08 mm (bottom). AcbSh, nucleus accumbens shell; DL, dorsolateral striatum; DM, dorsomedial striatum; LS, lateral septum; S, subiculum; SNc, substantia nigra, compact part; Tu, olfactory tubercle; VTA, ventral tegmental area; VL, ventrolateral striatum.

**Fig. 2.** Basal NT mRNA expression in DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice. Sections illustrate in situ hybridization with a probe against NT mRNA. A and B: nucleus accumbens shell and olfactory tubercle (bregma = 1.1 mm); C and D: lateral septum (bregma = 0.5 mm); E and F: SN/VTA (bregma = -3.08 mm); G and H: subiculum (bregma = -3.08 mm). In DAT<sup>-/-</sup> mice, the number of NT mRNA-expressing neurons was increased in the septum and the SN/VTA. ac, anterior commissure; AcbSh, nucleus accumbens shell; LS, lateral septum; LV, lateral ventricle; S, subiculum; SNc, substantia nigra, compact part; Tu, olfactory tubercle; VTA, ventral tegmental area. Scale bars: 0.25 mm.

**Fig. 3.** Effect of acute haloperidol (halo) on NT mRNA expression in the dorsal striatum of DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice. Sections illustrate in situ hybridization with a probe against NT mRNA in the dorsal striatum (bregma = 1.1 mm) of DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice treated with vehicle or haloperidol 1 or 5 mg/kg i.p., and sacrificed 6 h later. Haloperidol 1 mg/kg increased the number of hybridized neurons in the dorsomedial, dorsolateral, and ventrolateral striatum of DAT<sup>+/+</sup> mice, but was unable to stimulate NT gene expression in DAT<sup>-/-</sup> mice. In contrast, administration of haloperidol 5 mg/kg resulted in a pronounced increase of labeled neurons in both DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice. ac, anterior commissure; DL, dorsolateral striatum; DM, dorsomedial striatum; LV, lateral ventricle; VL, ventrolateral striatum. Scale bar: 1 mm.

**Fig. 4.** Effect of acute amphetamine (amph) on NT mRNA expression in DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice. Sections illustrate in situ hybridization with a probe against NT mRNA at the level of the fundus striati (bregma = 1.1 mm) of DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice treated with vehicle or amphetamine (10 mg/kg i.p.), and sacrificed 6 h later. Amphetamine increased the number of NT mRNA-expressing perikarya in both DAT<sup>+/+</sup> and DAT<sup>-/-</sup> mice. FStr, fundus striati. Scale bar: 0.25 mm.