# Characterization of behavioral response to amphetamine, tyrosine hydroxylase levels, and dopamine receptor levels in neurokinin 3 receptor knockout mice

Rebecca E. Nordquist*, Helene Savignac, Meike Pauly-Evers, Gaby Walker, Fred Knoflach, Edilio Borroni, Patricia Glaentzlin, Bernd Bohrmann, Jurg Messer, Laurence Ozmen, Anita Albientz and Will Spooren

**The neurokinin 3 ($NK_3$) receptor is a novel target under investigation for improvement of symptoms of schizophrenia, because of its ability to modulate dopaminergic signaling. To further understanding of the function of this receptor, sensitivity to dopaminergic stimuli and levels of dopaminergic receptors and tyrosine hydroxylase in $NK_3$ receptor knockout mice were studied. Knockout of the receptor was confirmed by lack of $NK_3$ protein and lack of electrophysiological responsivity of presumed dopaminergic neurons to senktide. $NK_3$ receptor knockout mice showed mild hyperlocomotion and deficits on the rotarod. $NK_3$ receptor knockout mice did not show significant differences in sensitivity to locomotor effects of acute amphetamine (0.3, 1, and 3 mg/kg subcutaneously) or significant alterations in sensitization to locomotor effects of amphetamine, but did show nonsignificant hyperreactivity to 1 mg/kg amphetamine and a nonsignificantly increased propensity to develop sensitization. A small decrease in $D_1$ receptor binding was seen in the dorsal striatum and olfactory tubercle, and a small decrease of in tyrosine hydroxylase in the olfactory tubercle, but no change was seen in $D_2$ receptor binding. Together, these results support a role for the $NK_3$ receptor in reactivity to dopaminergic stimuli, but the lack of robust changes indicates that the sensitivity to dopamine may be activity-dependent or benign in nature.** ***Behavioural Pharmacology*** **19:518–529 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

**Behavioural Pharmacology** 2008, **19**:518–529

**Keywords: amphetamine, dopamine, dopamine receptor, knockout, locomotor activity, neurokinin, psychostimulant sensitization, rotarod, tachykinin, tyrosine hydroxylase**

F. Hoffmann-La Roche Ltd., PRBD-N, Psychiatry Disease Area, CH-4070 Basel, Switzerland

Correspondence to Dr Will Spooren, F. Hoffmann-La Roche, Psychiatry Disease Area, Building 72-148, CH-4070 Basel, Switzerland
E-mail: will.spooren@roche.com

**Received** 10 October 2007 **Accepted as revised** 3 April 2008

## Introduction

Dopamine signaling in the limbic system is well recognized to play an important role in numerous psychiatric disorders. The neurokinin 3 ($NK_3$) receptor is located in various areas of the limbic system, including the striatum and amygdala (Ding *et al.*, 1996), as well as, importantly, on dopaminergic neurons in the midbrain (Whitty *et al.*, 1995, 1997; Lévesque *et al.*, 2006). This localization on dopaminergic neurons gave rise to the idea that the $NK_3$ receptor may have the potential to modulate dopaminergic release in limbic areas. Indeed, activation of the midbrain $NK_3$ receptors with the endogenous ligand neurokinin B or the highly specific agonist succinyl-[Asp6, Me-Phe8]SP(6-11) (senktide) causes increased dopamine release in the striatum and prefrontal cortex (Humpel *et al.*, 1991; Tremblay *et al.*, 1992; Marco *et al.*, 1998), as well as increased electrophysiological activity of dopaminergic neurons (Overton *et al.*, 1992; Nalivaiko *et al.*, 1997).

This effect of $NK_3$ receptor stimulation on dopaminergic neurons and dopamine release implies that the $NK_3$ receptor may provide a means for modulating the dopaminergic system without the strong adverse side effects produced by $D_2$ receptor antagonists generally used as antipsychotics. The $NK_3$ receptor has thus been increasingly recognized as a potential target for treatment of schizophrenia (Spooren *et al.*, 2005). Indeed, two $NK_3$ receptor antagonists have shown promise in clinical testing as antipsychotics: SR-142801 (osanetant) and SB-223412 (talnetant) (Spooren *et al.*, 2005). Although development of osanetant was stopped, potentially because of efficacy issues (Meltzer and Prus, 2006) and the discontinuation of clinical trials for talnetant was also announced in a press release from GlaxoSmithKline (Middlesex, UK), it should be noted that osanetant exhibited less than optimal oral bioavailability, and talnetant showed poor brain penetration in preclinical testing

*The present address of Rebecca E. Nordquist is Emotion and Cognition Program, Department of Farm Animal Health, Veterinary Faculty, Utrecht University, Marburglaan 2, 3584CN Utrecht, the Netherlands.
E-mail: r.e.nordquist@uu.nl

0955-8810 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins

DOI: 10.1097/FBP.0b013e32830cd7f5

(Spooren *et al.*, 2005). Notwithstanding, the fact that both of these compounds have been in clinical testing in schizophrenics highlights the interest in the $NK_3$ receptor as a target for drug development.

Investigation of effects of $NK_3$ receptor transmission has been seriously hindered by species differences between humans, rats and mice in the $NK_3$ receptor, that necessitate testing of pharmacological agents primarily in gerbils or guinea pigs. Current animal models are not optimized in these species. For further knowledge of the $NK_3$ receptor, a knockout mouse of the $NK_3$ receptor was created. Earlier studies from our own group demonstrated that these $NK_3$ knockout mice performed better in the delayed matching to position and active avoidance tasks (Nordquist *et al.*, 2008). These behavioral alterations in tasks known to be dopamine dependent, and the earlier demonstrated interactions of the $NK_3$ receptor with dopaminergic systems, led to the further examination of these mice from the perspective of dopaminergic action.

Given that the rationale for the use of the $NK_3$ antagonism in drug development lies largely in its interaction with dopaminergic systems, and the alterations in dopamine-dependent cognitive tasks in these $NK_3$ knockout mice, we conducted new studies to examine effects of knocking out the $NK_3$ receptor on dopamine-dependent motor behaviors, responsivity to (repeated) dopamine agonism, dopaminergic receptor and tyrosine hydroxylase levels, and baseline activity of dopaminergic neurons. Electrophysiological data, data on motor coordination, characterization of dopaminergic receptors, characterization of tyrosine hydroxylase levels, and behavioral responsivity to dopamine agonism are reported here for the first time in $NK_3$ knockout mice. Baseline locomotor activity results were compared with those from an earlier generated knockout line (Siuciak *et al.*, 2007).

## Methods

### Generation of neurokinin 3 knockout mice

$NK_3$ knockout mice were generated and bred as described below (and in Nordquist *et al.*, 2008).

#### *Cloning of the targeting vector*

A 3.7 kb (A) and a 2 kb fragment (B) of the Tacr3 ($NK_3$) mouse gene containing the 5′ region, exon 1 and a part of intron 1, were amplified by long-range PCR using the primer-combinations forward: CGGGG TACCA AATAA AGGAC CAACA GATCT GACAC, reverse: CGGCT CGAGT TGGAC ACCAG TAGTG ATGGC, and forward: CGGCT CGAGC TGCCA TCACT ACTGG TGTCC, reverse: CGCGG ATCCA TATAA AAGCT ATCTC AGATA GGG, respectively.

The primers introduced restriction-sites for subsequent cloning into a targeting vector. A loxP element was inserted 68 bp upstream of the exon 1 in fragment A by site directed mutagenesis. A diphtheria-toxin gene was cloned downstream of fragment B. A neo/thymidine kinase (TK) selection cassette with flanking loxP sites was introduced into the XhoI site downstream of exon 1.

#### *Generation of Tacr3tm1.1 mice*

The targeting vector was linearized and electroporated into embryonic stem (ES) cells derived from C57BL/6, which were maintained on subconfluent primary embryonic fibroblasts. Neomycin-resistant and diphtheria-toxin-selected colonies were isolated and expanded. The ES cells containing the homologous recombination event were determined by PCR with primers internal and external to the targeting construct. ES cell colonies, which underwent the correct recombination event (homologous integration of neo/TK-selection cassette and maintenance of loxP sites), were designated as Tacr3tm1.

One targeted tm1-clone was subjected to a second electroporation with a Cre-expression plasmid to remove the loxP-flanked exon 1 and neo/TK-selection cassette. Clones with a Cre-mediated deletion of either exon 1 and the selection-cassette together or the selection-cassette alone were identified by PCR-analysis and the resulting alleles were designated Tacr3tm1.1 and Tacr3tm1.2, respectively. Only Tacr3tm1.1 was injected into C57BL/6 blastocysts, which were implanted into pseudopregnant dams. Resulting chimeras were bred with C57BL/6 mice and offspring were selected for the transmission of the Tacr3tm1.1 allele. Mice used in the present studies were produced by intercrossing heterozygous mice for the Tacr3tm1.1 allele, then determination of progeny genotype by PCR of tail samples.

### Neurokinin 3 receptor western blot

#### *Preparation of brain tissue*

Four wild-type, two heterozygote, and two knockout mice were anesthetized by isoflurane inhalation [Forene (generic name = Isoflurane), Abbott, Rungis, France; 3–5% for induction], then decapitated. The brains were rapidly removed and repeatedly washed with PBS. CLB1-lysis buffer of 500 µl was freshly prepared by addition of phosphatase inhibitor cocktail II (at a dilution of 1 : 1000) and protease inhibitor cocktail tablets. The buffer and one brain hemisphere per mouse were homogenized in a micro-DOUNCE-homogenizator using 20 strokes, then stored at −20°C for further use.

#### *Bradford commassie protein concentration determination*

First, bovine serum albumin standard dilutions were diluted in lysis buffer at the range of 1.5 mg/ml, then to 0.025 mg/ml for the usage as internal calibrant. The samples were diluted and adjusted to a final volume of 25 µl with lysis buffer. Samples and 25 µl of each standard

dilution were mixed with 1 ml of the Bio-Rad Protein assay dry reagent dilution. Each solution of 200 µl was transferred on to a microplate and the absorption was determined at 595 nm.

#### *Protein detection with western blot analysis*

For western blot analyses, samples (25 µg/lane) were separated using 4–12% SDS-polyacrylamide gel electrophoresis (PAGE) (NuPAGE; Invitrogen, Carlsbad, California, USA) and transferred to Nitrocellulose membranes (Amersham Pharmacia Biotech, Uppsala, Sweden). Membranes were blocked in SuperBlock (Pierce, Rockford, Illinois, USA), followed by 5% nonfat-dry milk in 0.1% Tween/PBS for 30 min at room temperature. Incubation with the primary rabbit polyclonal anti-$NK_3$ antibody (ABR; 1 : 1000) in 0.5% milk solution occurred overnight at 4°C. Membranes were washed with 0.1% Tween/PBS and subsequently incubated with the secondary goat anti-rabbit HRP (Santa Cruz Biotechnolgy, Delaware Avenue, Santa Cruz, California, USA) in a dilution 1 : 2000 in 0.5% milk solution for 1 h at room temperature. Immunodetection was performed using the Lumi-Light Western Blotting Substrate (Roche Biochemicals, Roche Diagnostics GmbH, Mannheim, Germany). To evaluate equal loading of protein samples, stripped membranes were incubated with mouse anti-Tubulin antibodies (1 : 1000; Chemicon, Millipore Corporation, Billerica, Massachusetts, USA.), followed by the secondary goat anti-mouse HRP antibody (Santa Cruz Biotechnology).

### Electrophysiological responsivity of dopaminergic neurons to senktide

$NK_3$ knockout and wild-type mice (29–61 days old) were anesthetized with 2% isoflurane for 2 min and decapitated. Their brains were quickly removed and immersed in an ice-cold artificial cerebrospinal fluid (aCSF) containing NaCl, 119 mmol/l, KCl, 2.5 mmol/l, $CaCl_2$, 2.5 mmol/l, $MgCl_2$, 1.3 mmol/l, $NaH_2PO_4$, 1 mmol/l, $NaHCO_3$ 26.2, glucose, 11 mmol/l and bubbled with 95% $O_2$ – 5% $CO_2$ (pH 7.4). Horizontal slices (250 µm) containing the ventral tegmental area (VTA) were cut with a vibratome (VT1000S; Leica, Nussloch, Germany), transferred to aCSF (35°C) for 30 min and stored at room temperature in aCSF bubbled with 95% $O_2$ – 5% $CO_2$ for at least 1 h for equilibration. For electrophysiological experiments, a slice was transferred to the recording chamber (1 ml volume) and continuously perfused with bubbled aCSF at a rate of 2 ml/min and held at 35°C. Electrodes were pulled with a micropipette puller (Zeitz, Germany) from thin-wall borosilicate glass pipettes (1.5 mm outer diameter, Clark) and filled with aCSF (electrode resistance, 2.5–4 MΩ). The ventral tegmental area was identified as the region lying medial to the terminal nucleus of the accessory optic tract (Paxinos and Watson, 1986). Extracellular recordings were made from spontaneously active dopamine neurons. A neuron was considered dopaminergic if it had a regular firing pattern (0.5–2 Hz) a broad ( > 2 ms), triphasic action potential; and was sensitive to quinpirole (100 nmol/l), a dopamine receptor agonist (Grace and Onn, 1989). Extracellular signals were high-pass and low-pass filtered at 1 Hz and 2 kHz, respectively, with a Multiclamp 700A amplifier (Axon Instruments, Foster City, California, USA) and digitized at 10 kHz with the DigiData 1200 interface and pClamp 8.0 software (both Axon Instruments).

Stock solutions of the $NK_3$ agonist senktide (Tocris; Tocris Bioscience, Bristol, UK) and quinpirole hydrochloride (Sigma-Aldrich, St Louis, Missouri, USA) were made in $H_2O$ and diluted in aCSF to final concentrations shortly before being applied to the slice. To determine the firing frequency of a neuron, extracellular action potentials were detected by threshold search using the pClamp software during periods of 10 s. The mean firing rates were determined over a time period of 2–3 min in the absence and presence of a drug. The changes in firing rates induced by drugs were normalized as a percentage of control and expressed as mean ± SE.

### Behavioral testing

For all experiments, with the exception of rotarod performance, male and female mice were tested. No significant interactions were found between sex and task performance, therefore all data in figures are depicted with both sexes grouped. Where relevant, additional statistics on sex are provided in the results section.

#### *Neurological assessment*

All mice used in behavioral testing were subjected to a neurological assessment in which body weight and body temperature, general appearance, reflexes, strength, responsivity to various stimuli, and behavior in an open field were observed for any obvious abnormalities.

#### *Motor coordination*

Motor coordination assessed by testing male $NK_3$ knockout and wild-type mice ($n = 14$ per genotype) on a rod rotating at 16 and 32 RPM for either five trials or until criterion of 120 s on the rod was achieved. The mean time on the rod over the number of trials completed per mouse per speed was calculated. Across the groups, medians and intraquartile ranges were calculated. A Mann–Whitney *U* test was used to statistically compare the two genotypes.

#### *Locomotor activity*

*Baseline*

Wild-type ($n = 47$), heterozygote ($n = 70$), and knockout ($n = 50$) animals were placed a large open field ($42 \times 42 \times 30$ cm, w × l × h) of a VersaMax System (AccuScan Instruments Inc, Columbus, Ohio, USA) locomotor activity recording setup, and allowed to move freely within the open field for 90 min, whereas total distance traveled was calculated by the number of infrared beam breaks.

Activity was measured in 30-min bins across the 90 min session. A repeated-measures analysis of variance (ANOVA) using genotype and sex as factors was used for statistical comparisons, followed by a Newman-Keuls post-hoc test when appropriate.

*Response to amphetamine*

In three separate experiments, responses to 0.3, 1, and 3 mg/kg of D-amphetamine were tested. For measurement of responses to 0.3 mg/kg ($n = 32$–$37$ per genotype per drug treatment) or 1 mg/kg ($n = 35$–$38$ per genotype per drug treatment) of amphetamine, animals were first habituated to an open field ($20 \times 20 \times 30$ cm, w × l × h) for 60 min, then injected with amphetamine or vehicle subcutaneously, after which recording of locomotor activity commenced immediately. For measurement of responses to 3 mg/kg of D-amphetamine (Roche, Basel, Switzerland; $n = 31$–$34$ per genotype per drug treatment), animals were injected in the home cage, then after a 15-min pretreatment interval were placed in a large open field ($42 \times 42 \times 30$ cm, w × l × h). For all experiments, total distance traveled was recorded for 60 min in three blocks of 20 min, then tested statistically using a repeated-measures ANOVA with genotype, drug treatment, and sex as between-subjects and time as within-subjects factors, followed by *t*-tests comparing vehicle to amphetamine-injected groups within each genotype.

*Amphetamine sensitization*

Wild-type, heterozygote, and knockout mice were pretreated with 3 mg/kg of amphetamine or vehicle once daily on four consecutive days, followed by a drug abstinence period of 24–26 days. On the test day, half of the amphetamine-pretreated animals were challenged with 1 mg/kg of amphetamine (amphetamine/amphetamine group), and the other half with vehicle (amphetamine/vehicle group). Furthermore, half of the vehicle-pretreated animals were challenged with amphetamine (vehicle/amphetamine group) and the remainder with vehicle (vehicle/vehicle group ($n = 8$–$11$ per genotype per drug treatment group). Animals were immediately placed in an open field ($20 \times 20 \times 30$ cm, w × l × h) and total distance traveled was recorded for 90 min in 18 blocks of 5 min, then tested statistically using a repeated-measures ANOVA with genotype, pretreatment and challenge as between-subjects and time as within-subjects factors, followed by a Newman-Keuls post-hoc test.

### Histological characterization of dopaminergic system

#### *Receptor-binding studies*

Male wild-type and knockout mice were killed, and their brains rapidly removed, frozen in dry ice powder and stored at $-70^{\circ}$C ($n = 6$ per genotype for striatal areas; $n = 8$ per genotype for medial prefrontal cortex). Coronal sections of 10 µm were cut and thaw-mounted on adhesion glass slides (Paul Marienfeld GmbH, Lauda-Koenigshofeh, Germany). Dopamine $D_1$ receptors were measured essentially according to the procedure of Wong *et al.* (2003). Briefly, brain sections were incubated for 60 min at room temperature in a buffer containing 120 mmol/l NaCl, 5 mmol/l KCl, 1 mmol/l $MgCl_2$, 2 mmol/l $CaCl_2$, 50 mmol/l Tris–HCl buffer pH 7.4, 1 nmol/l [3H]-SCH23390 1 nmol/l (NET 930 Perkin Elmer, Switzerland), and 1 µmol/l mianserin hydrochloride (Sigma-Aldrich, Switzerland) to block serotoninergic receptors (Millan *et al.*, 2001). Pifluthixol of 1 µmol/l was used to determine nonspecific binding.

$D_2$ receptors were measured following instructions of Siemiatkowski *et al.* (2004). Brain slices were preincubated in 50 mmol/l Tris–HCl, 120 mmol/l NaCl, 5 mmol/l KCl, 2 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, and 0.1% ascorbic acid buffer (pH 7.4) for 20 min at room temperature, then incubated for 45 min with [3H]-Raclopride 2 nmol/l (NET-975, Perkin Elmer, Switzerland) in the same buffer. Nonspecific binding was determined with 10 µmol/l butaclamol.

For all autoradiographic studies brain sections were rinsed three times in cold buffer and once in cold distilled water before drying. Slide-mounted sections together with [3H]-microscale (RPA 510, GE Healthcare, Switzerland) were exposed to a Fuji Imaging plate (BAS TR 2025, FujiFilm, Switzerland) for 5 days. The imaging plate was then scanned in a FujiFilm image plate scanner (BAS-5000, Bucher Biotech, Switzerland). Densities in the medial prefrontal cortex, caudate putamen, nucleus accumbens, and olfactory tubercle in four sections from each animal were measured using the MCID Analysis program (InterFocus, Mering, Germany). Statistical significance of averages per animal per brain area was determined using a two-tailed *t*-test for independent measures.

#### *Immunohistochemistry*

$NK_3$ wild-type and knockout mice ($n = 6$ per genotype) were perfusion fixed with 4% formaldehyde in PBS. Coronal sections of the brain were cut at 10 µm using a Leica CM3050 S cryostat (Leica Microsystems, Wetzlar, Germany). An indirect immunofluorescence staining procedure was used to reveal tyrosine hydroxylase immunoreactivity. Briefly, a rabbit primary antibody against tyrosine hydroxylase (Chemicon International Inc., Temecula, California, USA) was applied at 1 : 500 dilution for 1 h and detected using an affinity-purified goat anti-rabbit IgG conjugated to cyanine-3 (Cy3; Jackson ImmunoResearch, West Baltimore Pike West Grove, Pennsylvania, USA) at 1 : 100 dilution for 1 h.

For relative quantification of the difference in tyrosine hydroxylase expression by immunostaining, brain sections were scanned with a GenePix Personal 4100A microarray

scanner (Molecular Devices, California, USA). Parameter settings were adjusted for excitation wavelength of 532 nm (Cy3 filter) at single line averaging with a gain setting to acquire fluorescence intensities in the effective dynamic range of the photomultiplier tube and kept constant throughout the measurements to allow for quantitative intensity comparison.

## Results

### Western blot of neurokinin 3 protein in wild-type, heterozygote, and knockout mice

The knockout mice did not show any evidence of $NK_3$ protein in the brain, in contrast with wild-type and heterozygote littermates, as shown in Fig. 1. In wild types and heterozygotes, the expected bands were seen at 52 kDa for $NK_3$ protein, and for all animals the expected band at 50 kDa was seen for tubulin. This was in accordance with the PCR results from tail confirming a lack of $NK_3$ RNA in tail samples.

### Electrophysiological responsivity of dopaminergic neurons to senktide

In the VTA, extracellular, single-unit recordings revealed that mean firing frequencies of presumed dopaminergic neurons from wild-type and $NK_3$ knockout mice were not statistically different, yielding values of $1.75 \pm 0.21$ Hz ($n = 9$) and $1.40 \pm 0.21$ Hz ($n = 12$; $P > 0.15$), respectively (Fig. 2). In nine of 12 neurons from wild-type mice, senktide (100 nmol/l) produced an increase in firing frequency up to $175 \pm 21\%$ of control. In contrast, the firing frequencies of neurons recorded from $NK_3$ knockout mice in the presence of senktide (100 nmol/l) were not different from control ($97 \pm 1\%$; $n = 12$). Thus, a saturating concentration of the selective $NK_3$ agonist senktide excited neurons of the VTA of wild-type mice only.

### Neurological assessment

No abnormalities were observed in the body temperature or general appearance of the knockout mice, their responses to the stimuli presented, or their reflexes. A two-way ANOVA showed a significant effect of genotype [$F(2,69) = 8.24$, $P < 0.05$], sex [$F(1,69) = 609.38$, $P < 0.001$], and genotype × sex interaction [$F(2,69) = 8.21$, $P < 0.05$], on body weight. Female $NK_3$ knockout mice and heterozygote mice were heavier than their wild-type cohorts (knockout: $20.2 \pm 0.4$ g, heterozygotes: $20.1 \pm 0.26$; wild-type: $18.3 \pm 0.8$; $P < 0.05$ for wild-type vs. knockout and wild-type vs. heterozygotes), but no significant differences were seen between the genotypes in male mice (knockout: $27.8 \pm 0.6$ g, heterozygotes: $25.8 \pm 0.4$; wild-type: $25.2 \pm 0.4$, NS).

### Motor coordination

The $NK_3$ knockout mice showed no impairment on the slower setting (16 RPM) of the rotarod task, and a mild but significant impairment on the faster setting ($Z = 2.30$, $P < 0.05$) (Fig. 3).

### Locomotor activity

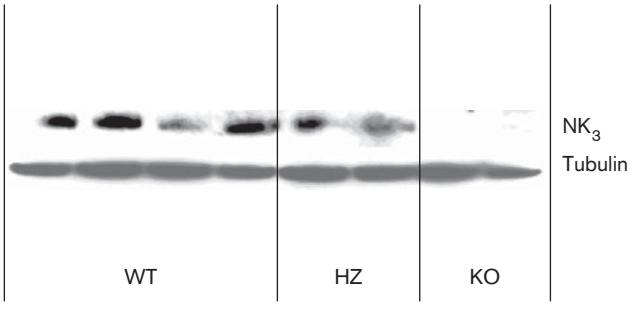
#### *Baseline*

$NK_3$ knockout mice showed significantly higher baseline levels of locomotor activity ($15890 \pm 801$ cm) than their wild-type littermates ($13571 \pm 607$ cm) during the 90-min test period [main effect of genotype: $F(2,161) = 4.43$, $P < 0.05$; post-hoc wild-type vs. knockout: $P < 0.05$; Fig. 4a]. Heterozygotes showed an intermediate level of locomotor activity (heterozygotes: $15025 \pm 558$ cm) significantly differing from wild-type ($P < 0.05$) but not knockout animals. No significant effects of sex [$F(1,161) = 3.04$, NS] or sex × genotype interaction [$F(2,161) = 0.89$, NS] were observed. The decrease in activity seen across the session is reflected in the main effect of time [$F(2,322) = 445.5$, $P < 0.001$]. No interaction between time with any of the other factors was observed [time × genotype: $F(4,322) = 1.26$; time × sex: $F(2,322) = 1.78$; time × genotype × sex: $F(4,322) = 0.52$; all NS].

#### *Response to amphetamine*

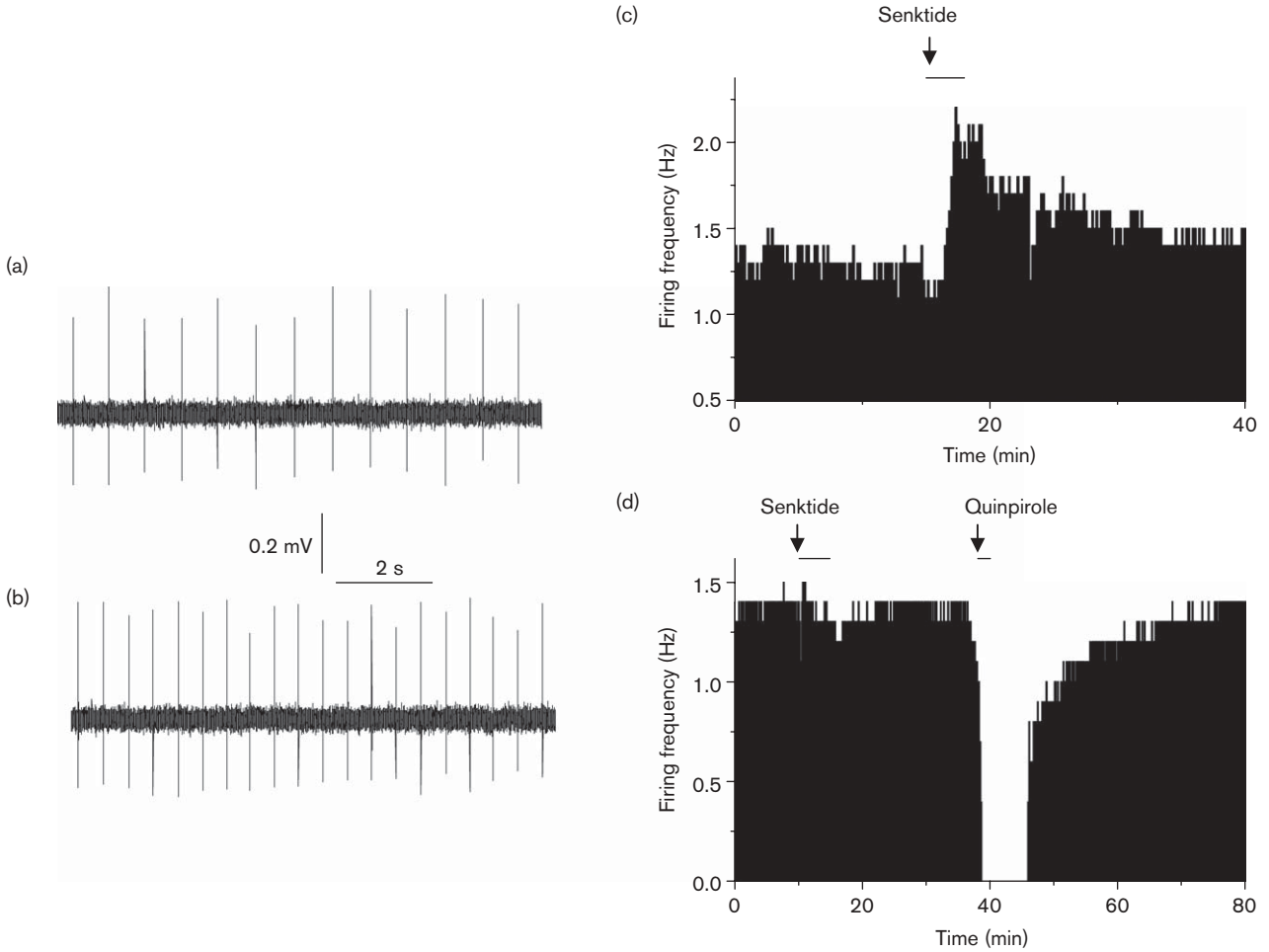
Treatment with 0.3 mg/kg of amphetamine after a habituation period had no significant effect on locomotor activity overall [$F(1,200) = 0.01$, NS; Fig. 4b] and no effect of genotype [$F(2,200) = 0.40$, NS] or genotype × drug treatment interactions [$F(2,200) = 0.68$, NS] were seen. No significant effects of sex [$F(1,200) = 1.16$, NS], sex × drug [$F(1,200) = 0.0.01$, NS] or sex × genotype × drug [$F(2,200) = 2.23$, NS] were observed. A decrease in activity across the time period measured was observed [$F(2,400) = 68.21$, $P < 0.001$], as well as a significant interaction between time and drug treatment [$F(2,400) = 14.65$, $P < 0.001$] and a marginally significant interaction between time, drug treatment, and sex [$F(2,400) = 3.03$, $P = 0.05$]. No significant interactions for time × genotype [$F(4,400) = 1.36$, NS], time × sex [$F(2,400) = 0.35$, NS], time × genotype × drug [$F(4,400) = 0.53$, NS], time × genotype × sex [$F(4,400) = 0.37$, NS] or time ×

**Fig. 1**




Western blot demonstrating neurokinin 3 ($NK_3$) receptor protein in wild-type (WT) and heterozygote (HZ) animals, and a lack of $NK_3$ protein in knockout (KO) animals. Top band represents $NK_3$ protein, bottom band represents tubulin as a control marker.

**Fig. 2**




Senktide enhances the firing rate of a presumed dopaminergic ventral tegmental area neuron from a neurokinin 3 ($NK_3$) wild-type mouse. Spontaneous action potentials recorded extracellularly in presumed dopaminergic neurons in $NK_3$ knockout mice in the absence (a) and presence (b) of 100 nmol/l senktide. Firing rate of presumed dopaminergic neuron in wild-type (c) and knockout (d) mouse plotted as a function of time. Each vertical bar represents the average firing frequency over a 10 s interval. The horizontal bar in (c) and leftmost horizontal bar in (d) indicates the duration of bath application of 100 nmol/l senktide. The right-hand horizontal bar in (d) indicates bath application of quinpirole.

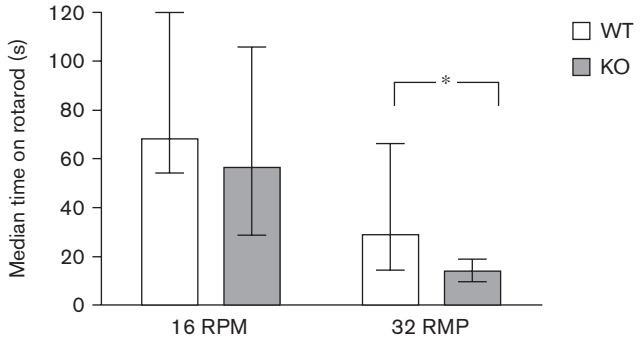
genotype × sex × drug treatment were observed [$F(4,400) = 0.576$, NS].

In animals tested after an injection of 1 mg/kg of amphetamine after a habituation period, an overall increase in locomotor activity was observed, seen as a significant main effect of injection in the repeated-measures ANOVA [$F(1,199) = 25.51$, $P < 0.001$; Fig. 4c]. No significant main effect of genotype [$F(2,199) = 2.01$, NS] or genotype × drug treatment interaction was observed [$F(2,199) = 1.08$, NS]. The increase in locomotor activity induced by amphetamine was more marked and reached statistical significance in $NK_3$ knockout mice in exploratory post-hoc testing ($P < 0.001$). By comparison, smaller increases were seen in the wild-type and heterozygote animals. In wild-type and heterozygote animals, amphetamine-treated animals did not differ significantly from their vehicle-treated controls in post-hoc testing.

Injection of 3 mg/kg caused an increase in locomotor activity which was highly significant [$F(1,186) = 278.46$, $P < 0.001$, Fig. 4d]. No significant effect of genotype [$F(2,186) = 0.50$, NS] or genotype × drug treatment interaction was observed [$F(2,186) = 0.08$, NS]. Post-hoc testing showed significant differences for knockout, wild-type, and heterozygote animals between amphetamine-treated and vehicle-treated animals ($P < 0.05$ for all three comparisons).

A significant main effect of sex was observed in the animals treated with 3 mg/kg of amphetamine,

**Fig. 3**



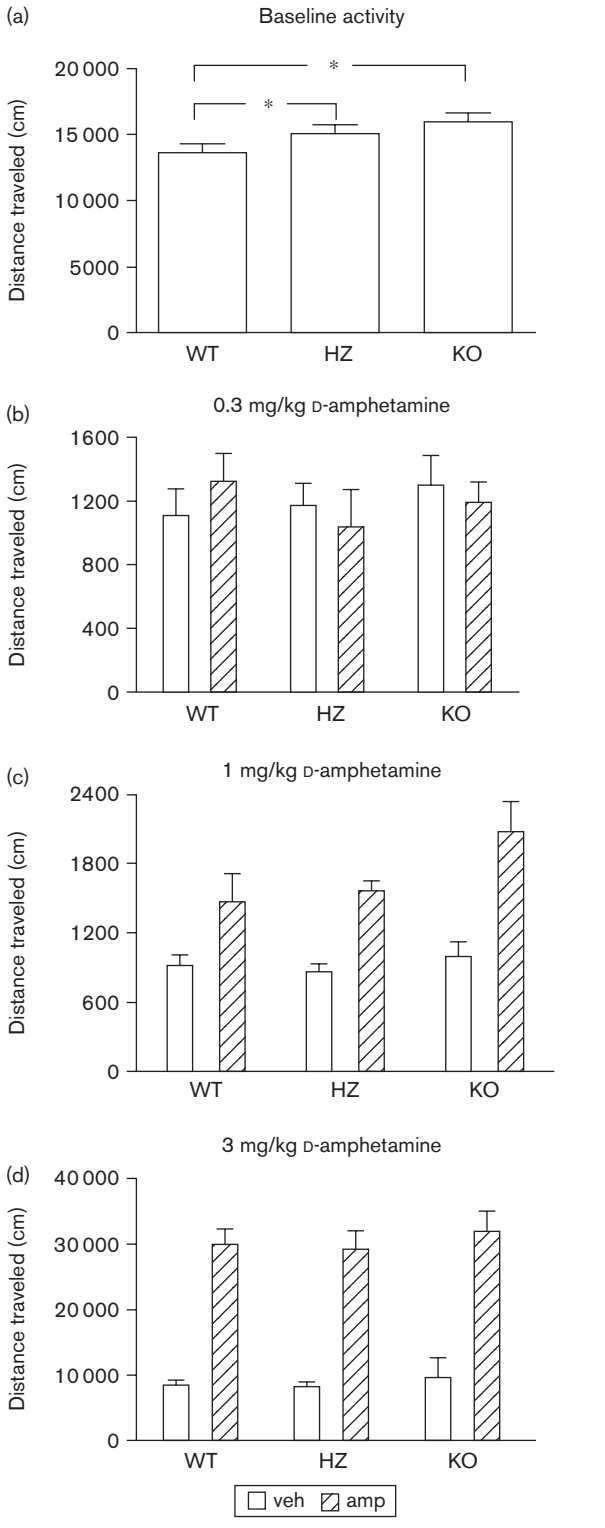

Rotarod performance of knockout (KO), heterozygote, and wild-type mice (WT). KO mice showed no significant impairment on the less difficult setting (16 RPM, left group of bars). A mild but significant impairment was seen on the more difficult 32-RPM setting (right two bars). Bars represent median performance per genotype; white bars represent WT mice, gray bars indicate KO mice. Error bars indicate interquartile ranges. *$P<0.05$ in Mann–Whitney $U$ testing.

with the female mice (21 471 ± 1582 cm traveled) more active than the male mice [18 061 ± 1256 cm traveled; $F(1,186) = 5.23$, $P < 0.05$], though there was no significant sex × genotype [$F(2,186) = 0.43$, NS] or genotype × sex × drug treatment [$F(2,186) = 1.24$, NS] interaction. Sex did interact with drug treatment [$F(1,186) = 4.12$, $P < 0.05$], in female mice treated with amphetamine traveling greater distance than male mice (female: 11 076 ± 429, male: 9211 ± 429; $P < 0.05$) but no difference between the sexes when treated with vehicle (female: 2990 ± 423, male: 2877 ± 425, NS). A significant effect of time [$F(2,372) = 204.28$, $P < 0.001$], with significant interactions between time and sex [$F(2,372) = 20.67$, $P < 0.001$], time and drug treatment [$F(2,372) = 47.29$, $P < 0.001$], and time, sex, and drug treatment was seen [$F(2,372) = 5.57$, $P < 0.01$], but no significant interactions between time and genotype [$F(4,372) = 0.49$, NS], time, genotype and sex [$F(4,372) = 0.16$, NS], time, genotype and drug treatment [$F(4,372) = 0.44$, NS] or time, genotype, drug treatment and sex [$F(4,372) = 0.14$, NS].

### *Amphetamine sensitization*

The drug treatment protocol was successful in inducing sensitization of the locomotor stimulant effect of 1 mg/kg of amphetamine, as evidenced by the significant main

**Fig. 4**



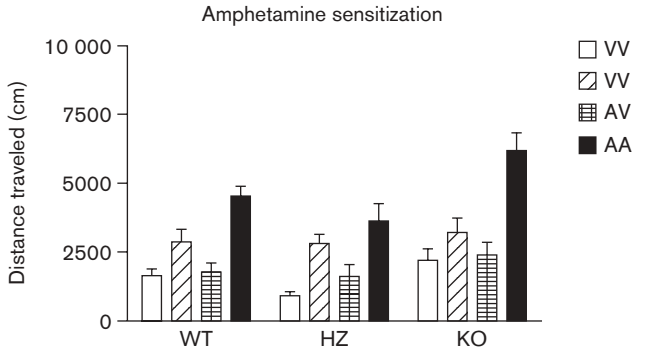

Locomotor activity of $NK_3$ knockout (KO), heterozygote (HZ) and wild-type mice (WT). For all graphs, $y$-axis represents distance traveled in centimeters and bars are grouped by genotype on $x$-axis. (a) Baseline activity in an open field measured for 90 min measured as total distance traveled. (b–d) Total distance traveled during a 60-min period after injection of amphetamine at 0.3 (b), 1 (c), or 3 (d) mg/kg subcutaneously and a 15-min pretreatment period. A 60-min habituation period preceded the 0.3 and 1 mg/kg treatment, thus activity in vehicle-treated groups is lower for these experiments. Open bars indicate vehicle-treated animals; hatched bars represent amphetamine-treated animals. *$P<0.05$ in post-hoc testing. Error bars represent SEM.

effects of pretreatment [$F(1,95) = 15.66$, $P < 0.001$], challenge [$F(1,95) = 59.71$, $P < 0.001$], and a significant pretreatment × challenge interaction [$F(1,95) = 7.09$, $P < 0.01$] (Fig. 5). Furthermore, there was a significant main effect of genotype [$F(2,95) = 7.59$, $P < 0.001$], with the knockout mice significantly more active overall than the wild type or heterozygotes in post-hoc testing ($P < 0.05$).

Sensitization was evident in knockout mice, with the amphetamine/amphetamine group differing significantly from all other treatment groups of knockouts in exploratory post-hoc testing, though the interaction between genotype, pretreatment, and challenge was not significant [$F(2,95) = 1.96$, NS]. Sensitization was not observed in wild-type or heterozygote mice, as seen in the lack of a significant difference between amphetamine/amphetamine and vehicle/amphetamine groups in both genotypes in post-hoc testing. Furthermore, amphetamine/amphetamine-treated knockout mice traveled significantly greater distances than the amphetamine/amphetamine-treated wild-type or heterozygote animals in exploratory post-hoc testing.

The time course of locomotor activation differed between the various treatment groups. An overall significant main effect of time [$F(17,1615) = 37.22$, $P < 0.001$], as well as significant interactions between time × pretreatment [$F(17,1615) = 16.85$, $P < 0.001$], time × challenge [$F(17,1615) = 39.36$, $P < 0.001$], time × genotype × pretreatment [$F(34,1615) = 1.46$, $P < 0.05$], and time × pretreatment × challenge were observed [$F(17,1615) = 14.61$, $P < 0.001$]. No significant time × genotype × challenge [$F(34,1615) = 1.08$, NS] or time × pretreatment × challenge × genotype [$F(34,1615) = 1.29$, NS] interactions were observed.

**Fig. 5**




Locomotor activity of $NK_3$ knockout (KO), heterozygote (HZ), and wild-type mice (WT) in an amphetamine sensitization paradigm. Mice were pretreated with 3 mg/kg amphetamine for 4 days, followed by 24–26 days of abstinence, and then challenged with either 1 mg/kg amphetamine (AA) or vehicle (AV). Pretreatment control groups were treated with vehicle for 4 days, then challenged with either 1 mg/kg amphetamine (VA) or vehicle (VV). Error bars represent SEM.

### Histological characterization of mesolimbic dopamine system

#### *Dopamine receptor autoradiography*

Within the areas measured, a small but significant decrease was observed in $D_1$ receptor-binding levels in the caudate putamen [$t(10) = 2.86$, $P < 0.05$] and olfactory tubercle [$t(10) = 2.70$, $P < 0.05$], whereas levels of $D_1$ receptor binding in the nucleus accumbens [$t(9) = 1.43$, NS; Fig. 6c and d] and medial prefrontal cortex were unaffected by genotype [$t(14) = 1.39$, NS; Figs 6a, b and 7].

$D_2$ receptor-binding levels did not differ significantly in any of the areas measured [Fig. 7; caudate putamen: $t(10) = 0.05$, NS; nucleus accumbens: $t(10) = 1.09$, NS; olfactory tubercle: $t(10) = 0.54$, NS; Figs 6c, d and 7].

#### *Tyrosine hydroxylase expression*

A significant decrease in tyrosine hydroxylase staining intensity, quantified as average fluorescent intensities, was observed in the olfactory tuberculum (wild-type 430.0 ± 12.2, knockout 348.7 ± 14.7; $t(10) = 4.26$, $P < 0.01$; Fig. 6g and h). No significant differences were found in levels of tyrosine hydroxylase between the $NK_3$ wild-type and knockout mice in the caudate putamen [wild-type: 343.7 ± 12.0, knockout 318.2 ± 9.1 $t(10) = 1.70$, NS] or nucleus accumbens [wild-type 341.2 ± 13.4, knockout 317.4 ± 10.4; $t(10) = 1.40$, NS].

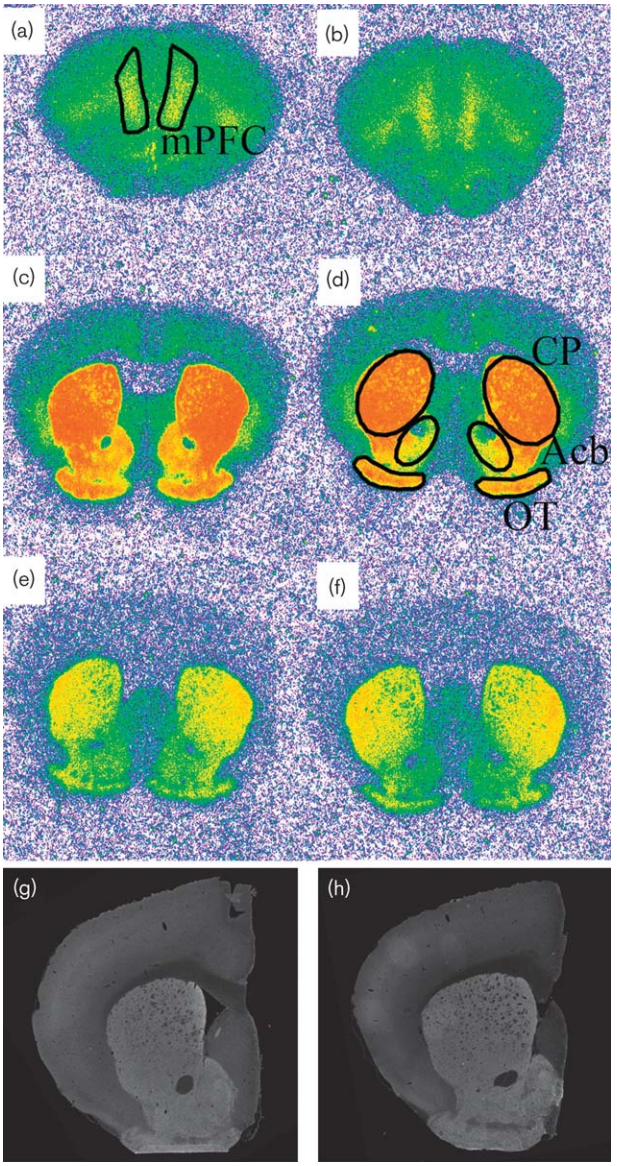
## Discussion

Knockout of the $NK_3$ receptor led to mild hyperlocomotion in a novel environment and mild deficits in rotarod performance. The $NK_3$ knockout mice did not show statistically significant alterations in sensitivity to amphetamine or in propensity to develop amphetamine sensitization. Furthermore, levels of the dopamine $D_1$ receptor were slightly decreased in the dorsal striatum and olfactory tuberculum, and tyrosine hydroxylase in the olfactory tubercle was also slightly decreased in $NK_3$ knockout mice. In contrast, $D_1$ receptor binding in the accumbens and $D_2$ receptor-binding levels in the dorsal striatum, accumbens, olfactory tubercle, and prefrontal cortex were unchanged, and no effect of the knockout was seen on tyrosine hydroxylase levels in the nucleus accumbens or dorsal striatum.

### Neurokinin 3 protein levels and electrophysiological response to senktide

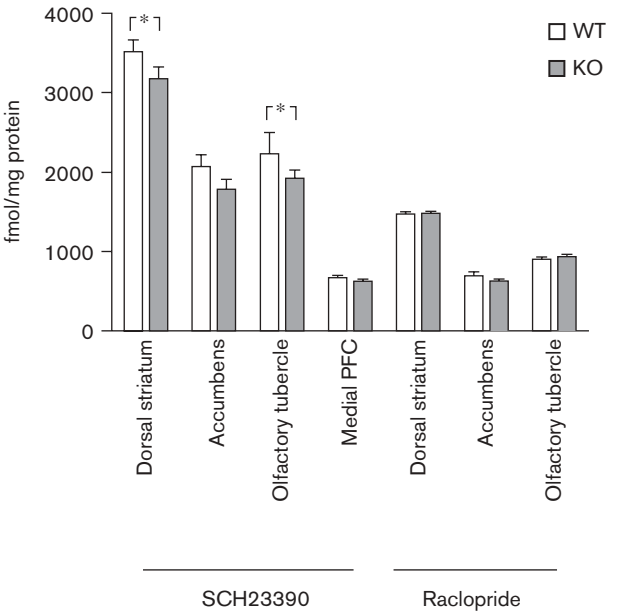
The knockout of the $NK_3$ receptor was confirmed both at protein level by western blotting, and functionally using the $NK_3$ receptor agonist senktide to stimulate presumed dopaminergic neurons in the ventral tegmental area. The $NK_3$ agonist senktide has been demonstrated to increase

**Fig. 6**




Representative sections of wild type (a, c, e, and g) and knockout (b, d, f, and h) mice radiolabeled for dopamine $D_1$ (a–d) and $D_2$ (e and f) receptors and immunostained for tyrosine hydroxylase (g and h). Areas quantified are as indicated in (a) for medial prefrontal cortex (mPFC) and 4D for caudate putamen (CP), nucleus accumbens (Acb) and olfactory tubercle (OT).

**Fig. 7**




Quantification of binding levels of the $D_1$ receptor ligand SCH23390 and the $D_2$ receptor ligand raclopride. Bars are grouped by ligand and brain area quantified on the *x*-axis. *Y*-axis represents fmol/mg of protein. Open bars represent wild-type (WT) mice, and gray bars represent knockout (KO) animals. $*P<0.05$ in post-hoc testing. Error bars represent SEM.

firing of electrophysiologically identified neurons in the midbrain in guinea pig (Nalivaiko *et al.*, 1997) and rat (Overton *et al.*, 1992; Seabrook *et al.*, 1995), an effect which presumably underlies the dopamine release observed in the striatum after senktide infusion into the VTA (Marco *et al.*, 1998). This study is, to our knowledge, the first demonstration that this senktide-induced activity in presumed dopaminergic neurons also occurs in mice. Furthermore, the lack of activity in knockout mice with a confirmed lack of the $NK_3$ receptor further validates the specificity of senktide-induced activation of presumed dopaminergic neurons to the $NK_3$ receptor.

### Mild hyperlocomotion and impairment of neurokinin 3 knockout mice on rotarod performance

Behaviorally, the $NK_3$ knockout mice showed a small increase in locomotor activity and impairment on the more difficult setting of the rotarod task. Another line of $NK_3$ knockout mice generated by Siuciak *et al.* (2007) did not show differences in locomotion. This discrepancy between the present results and those of Siuciak *et al.* (2007) could be attributable to procedural differences, specifically the relatively large number of subjects in this study of spontaneous locomotion ($n = 47$–$70$ per genotype) and the somewhat shorter time period measured in this study (90 min compared with 180 min in Siuciak *et al.*, 2007). This difference in locomotion is not the only difference found between the $NK_3$ knockout line described in this study and the line generated by Siuciak and colleagues; these lines actually show opposite effects in cognitive tasks (Siuciak *et al.*, 2007; Nordquist *et al.*, 2008). Differences observed in performance between the lines could be because, aside from the above-mentioned small differences in test protocol, of differences in the generation of the $NK_3$ knockouts. The knockouts described in this study were generated using C57BL/6 ES cells, whereas the line generated by Siuciak *et al.* was

generated using 129P2/OlaHsd ES cells and injection into C57BL/6J blastocysts. C57BL/6 and 129P2/OlaHsd mice show strongly divergent behavior, including (but not limited to) locomotion (Jacobson and Cryan, 2007). Any remaining flanking genes from the 129P2/OlaHsd line could influence behavior in the line described by Siuciak and colleagues, explaining the differences between the lines. This indicates that strain differences may play an important role in effects of $NK_3$ receptor knockout on phenotype, and thus potentially in $NK_3$ receptor antagonism.

The pattern of impairment observed on the rotarod, with impairment only at the highest rotations per minute setting, is often seen with dopamine $D_2$ receptor antagonists, and after acute treatment with atypical antipsychotics. Dopamine $D_2$ receptor knockouts also display deficits on the rotarod (Fowler *et al.*, 2002), but these deficits are considerably more profound than the effect observed in this study. Additionally, rotarod deficits after acute treatment with atypical or typical antipsychotics are usually seen in combination with hypolocomotion, whereas the $NK_3$ knockout line in the present studies showed a hyperlocomotion in response to a novel environment. This indicates that the animals may show motor coordination deficits independent of locomotor activity. Owing to the species difficulties involved in testing $NK_3$ antagonists and the use of nontraditional laboratory animals (primarily gerbils and guinea pigs) for profiling $NK_3$ antagonists, it is not known whether these compounds produce rotarod deficits when administered acutely. It would be of interest to see if it is practically feasible to test rotarod performance in gerbils, given the high affinity for $NK_3$ receptor antagonists for the gerbil receptor, and to examine the effects of acute $NK_3$ antagonism on motor behaviors.

#### Behavioral sensitivity to amphetamine

The interaction between drug and genotype was not significant at any dose of amphetamine tested, but results at the mid-range dose were suggestive of an enhanced response to 1 mg/kg in the $NK_3$ knockout mice. Indeed, explorative statistical testing comparing the various groups showed a significant difference between $NK_3$ knockout mice treated with vehicle and 1 mg/kg amphetamine, a difference which was not seen with wild-type or heterozygote animals. It should be noted that although the $NK_3$ knockout mice also show a mild hyperactivity in a novel environment without drug, the response to 1 mg/kg amphetamine occurred after a habituation period, after which no differences were seen between the genotypes in vehicle-treated animals.

The data suggestive of enhanced locomotor response to amphetamine in the knockout animals fit well with data on effects of chronic antipsychotic treatment. When given acutely, antipsychotic drugs inhibit locomotor activity caused by dopamine agonists. In a similar vein, acute antagonism of the $NK_3$ receptor with osanetant in rats led to attenuation of cocaine-induced locomotor activity (Jocham *et al.*, 2006). After chronic treatment with antipsychotics, psychostimulant-induced activity is, however, no longer reversed but actually potentiated under certain treatment regimens (Marin *et al.*, 1993; Meng *et al.*, 1998; Andersen and Pouzet, 2001). As knockout animals chronically lack the $NK_3$ receptor, the knockout state may be more comparable with chronic rather than acute antipsychotic treatment. The opposing effects observed between responding to a dopamine agonist after acute treatment with an $NK_3$ antagonist observed in the study by Jocham *et al.* (2006) and knockout of the $NK_3$ receptor in this study may be because of neuroadaptations that take place after chronic lack of the $NK_3$ receptor.

As with acute amphetamine, the results of $NK_3$ knockout mice were suggestive of hypersensitivity to the effects of repeated amphetamine. Thus, although the interaction between genotype, pretreatment and challenge was not significant, precluding conclusive statements about increased propensity to sensitization, the exploratory post-hoc tests strongly suggest a mild hypersensitivity. The natural ligand of the $NK_3$ receptor, neurokinin B, has also been found to be reduced in the striatum, after repeated cocaine administration on a schedule that would produce sensitization (Che *et al.*, 2006), another indicator that the neurokinin system may be involved in psychostimulant sensitization. An eventual enhanced sensitivity of $NK_3$ knockout mice to amphetamine sensitization may be an indicator of alterations in the dopaminergic system, as one of the hallmarks of amphetamine sensitization is enhanced dopaminergic release in the striatum in response to amphetamine. It is worth noting, however, that the *N*-methyl-D-aspartate (NMDA) glutamate receptor also plays a critical role in the development of sensitization (for review, see Vanderschuren and Kalivas, 2000 ). $NK_3$ receptors interact with NMDA receptors at the level of the spinal cord, influencing electrophysiological recordings of patterns resembling those that produce locomotion (Marchetti and Nistri, 2001). In a more complex interaction, $NK_3$ receptor antagonism reduces NMDA-evoked acetylcholine release in specific areas of the striatum in a dopamine-independent manner (Kemel *et al.*, 2002). Together these data suggest a possible role for dopamine–$NK_3$ or glutamate–$NK_3$ interactions in potentiation of sensitization.

#### Dopamine receptor and precursor levels are unaltered or mildly decreased in neurokinin 3 knockouts

Although the $NK_3$ receptor has been demonstrated to interact heavily with the dopaminergic system (for review, see Spooren *et al.*, 2005), this study demonstrated

no or only minimal effects of knocking out the $NK_3$ receptor on dopamine receptor levels and on levels of the dopamine synthetic enzyme tyrosine hydroxylase. This stands in contrast to increases in levels of dopamine $D_2$ receptors in the striatum caused by chronic treatment with antipsychotics, which work as dopamine $D_2$ receptor antagonists (Burt *et al.*, 1977; Piazza *et al.*, 1990; Florijn *et al.*, 1997; Huang *et al.*, 1997; Joyce, 2001). A possible explanation is that interactions of the $NK_3$ receptor with the dopaminergic system may be activity dependent. Thus, at the steady-state of a knockout animal without pharmacological challenge, no or minor long-term alterations in the dopaminergic system are seen, whereas a lack of $NK_3$ receptors affects acute responses to a dopamine-agonist challenge as seen in the locomotor response to amphetamine. Alternatively, the trend toward hyperresponsivity of the $NK_3$ knockouts to amphetamine could be because of other neurotransmitter systems such as the glutamatergic interactions described in the section above with reference to amphetamine sensitization, or serotonergic, cholinergic or noradrenergic systems, with which neurokinins also interact (Spooren *et al.*, 2005; Meltzer and Prus, 2006).

The small decrease in dopamine $D_1$ receptors is of interest in light of known interactions between dopamine $D_1$ receptors and neurokinin receptors. Acute $NK_3$ antagonism attenuates dopamine $D_1$ agonist-induced activity (Bishop and Walker, 2004), and senktide-induced cardiovascular responses are blocked by the $D_1$ receptor antagonist SCH23390, but not by the $D_2$ antagonist raclopride (Deschamps and Couture, 2005). Furthermore, it is also noteworthy that the $NK_1$ receptor has been demonstrated to influence $D_1$ receptor-mediated acetylcholine release indirectly (Anderson *et al.*, 1994), though the role of $NK_3$ in $D_1$-mediated neurotransmitter release has yet to be investigated.

In summary, this study provides the first description of effects of knockout of the $NK_3$ receptor on response to dopaminergic stimuli and characterization of dopaminergic receptors in these mice. The present results demonstrate that knockout of the $NK_3$ receptor produces hyperlocomotion, deficits in motor coordination, may cause enhanced behavioral responding to dopaminergic stimulation, and produces small decreases in expression of both dopamine $D_1$ receptors and tyrosine hydroxylase. Together, these data further support an interaction between $NK_3$ receptors and dopaminergic systems, with knockout of the $NK_3$ receptor producing subtle alterations in reactivity to dopaminergic stimuli and dopamine-dependent behavior. This subtle alteration provides a further basis for investigation of the $NK_3$ receptor as a potential target for antipsychotics, which could potentially provide changes in dopaminergic response to facilitate antipsychotic working, without the side effects produced by $D_2$ antagonism.

## Acknowledgements

The authors thank Dr J.G. Wettstein for his support during this project. All authors are employees at F. Hoffmann-La Roche, a pharmaceutical company engaged in research and development of drugs for central nervous system disorders.

## References

Anderson J, Kuo S, Chase T, Engber T (1994). Dopamine D1 receptor-stimulated release of acetylcholine in rat striatum is mediated indirectly by activation of striatal neurokinin1 receptors. *J Pharmacol Exp Ther* **269**:1144–1151.

Andersen MP, Pouzet B (2001). Effects of acute versus chronic treatment with typical or atypical antipsychotics on D-amphetamine-induced sensorimotor gating deficits in rats. *Psychopharmacology* **156**:291–304.

Bishop C, Walker PD (2004). Intranigral antagonism of neurokinin 1 and 3 receptors reduces intrastriatal dopamine D1 receptor-stimulated locomotion in the rat. *Brain Res* **1023**:126–133.

Burt D, Creese I, Snyder S (1977). Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. *Science* **196**:326–328.

Che FY, Vathy I, Fricker LD (2006). Quantitative peptidomics in mice: effect of cocaine treatment. *J Mol Neurosci* **28**:265–275.

Deschamps K, Couture R (2005). The ventral tegmental area as a putative target for tachykinins in cardiovascular regulation. *Br J Pharmacol* **145**: 712–727.

Ding YQ, Shigemoto R, Takada M, Ohishi H, Nakanishi S, Mizuno N (1996). Localization of the neuromedin K receptor (NK3) in the central nervous system of the rat. *J Comp Neurol* **364**:290–310.

Florijn WJ, Tarazi FI, Creese I (1997). Dopamine receptor subtypes: differential regulation after 8 months treatment with antipsychotic drugs. *J Pharmacol Exp Ther* **280**:561–569.

Fowler SC, Zarcone TJ, Vorontsova E, Chen R (2002). Motor and associative deficits in D2 dopamine receptor knockout mice. *Int J Dev Neurosci* **20**: 309–321.

Grace AA, Onn SP (1989). Morphology and electrophysiological properties of immunocytochemically identified rat dopamine neurons recorded in vitro. *J Neurosci* **9**:3463–3481.

Huang N, Ase AR, Hebert C, Van Gelder NM, Reader TA (1997). Effects of chronic neuroleptic treatments on dopamine D1 and D2 receptors: homogenate binding and autoradiographic studies. *Neurochem Int* **30**:277–290.

Humpel C, Saria A, Regoli D (1991). Injection of tachykinins and selective neurokinin receptor ligands into the substantia nigra reticulata increases striatal dopamine and 5-hydroxytryptamine metabolism. *Eur J Pharmacol* **195**:107–114.

Jacobson L, Cryan J (2007). Feeling strained? Influence of genetic background on depression-related behavior in mice: a review. *Behav Genet* **37**: 171–213.

Jocham G, Lezoch K, Muller CP, Kart-Teke E, Huston JP, De Souza Silva MA (2006). Neurokinin receptor antagonism attenuates cocaine's behavioural activating effects yet potentiates its dopamine-enhancing action in the nucleus accumbens core. *Eur J Neurosci* **24**:1721–1732.

Joyce JN (2001). $D_2$ but not $D_3$ receptors are elevated after 9 or 11 months chronic haloperidol treatment: influence of withdrawal period. *Synapse* **40**:137–144.

Kemel M-L, Perez S, Godeheu G, Soubrie P, Glowinski J (2002). Facilitation by endogenous tachykinins of the NMDA-evoked release of acetylcholine after acute and chronic suppression of dopaminergic transmission in the matrix of the rat striatum. *J Neurosci* **22**:1929–1936.

Lévesque M, Wallman MJ, Parent R, Sik A, Parent A (2006). Neurokinin-1 and neurokinin-3 receptors in primate substantia nigra. *Neurosci Res* **57**:362–371.

Marchetti C, Nistri A (2001). Neuronal bursting induced by $NK_3$ receptor activation in the neonatal rat spinal cord *in vitro*. *J Neurophysiol* **86**:2939–2950.

Marco N, Thirion A, Mons G, Bougault I, Le Fur G, Soubrie P, Steinberg R (1998). Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: an in vivo microdialysis approach in guinea pig. *Neuropeptides* **32**:481–488.

Marin C, Parashos SA, Kapitzoglou-Logothetis V, Peppe A, Chase TN (1993). D1 and D2 dopamine receptor-mediated mechanisms and behavioral supersensitivity. *Pharmacol Biochem Behav* **45**:195–200.

Meltzer H, Prus A (2006). NK3 receptor antagonists for the treatment of schizophrenia. *Drug Discov Today: Ther Strategies* **3**:555–560.

Meng Z-H, Feldpaush DL, Merchant KM (1998). Clozapine and haloperidol block the induction of behavioral sensitization to amphetamine and associated genomic responses in rats. *Mol Brain Res* **61**:39–50.

Millan MJ, Newman-Tancredi A, Quentric Y, Cussac D (2001). The 'selective' dopamine D1 receptor antagonist, SCH23390, is a potent and high efficacy agonist at cloned human serotonin$_{2C}$ receptors. *Psychopharmacology (Berl)* **156**:58–62.

Nalivaiko E, Michaud JC, Soubrie P, Le Fur G, Feltz P (1997). Tachykinin neurokinin-1 and neurokinin-3 receptor-mediated responses in guinea-pig substantia nigra: an in vitro electrophysiological study. *Neuroscience* **78**:745–757.

Nordquist RE, Delenclos M, Ballard TM, Savignac H, Pauly-Evers M, Ozmen L, Spooren W (2008). Cognitive performance in neurokinin 3 receptor knockout mice. *Psychopharmacology* **198**:211–220.

Overton P, Elliott PJ, Hagan RM, Clark D (1992). Neurokinin agonists differentially affect A9 and A10 dopamine cells in the rat. *Eur J Pharmacol* **213**:165–166.

Paxinos G, Watson C (1986). *The rat brain in stereotaxic coordinates*. 2nd ed. New York, USA: Academic Press.

Piazza PV, Calza L, Giardino L, Amato G (1990). Chronic thioridazine treatment differently affects DA receptors in striatum and in mesolimbo-cortical systems. *Pharmacol Biochem Behav* **35**:937–942.

Seabrook GR, Bowery BJ, Hill RG (1995). Pharmacology of tachykinin receptors on neurones in the ventral tegmental area of rat brain slices. *Eur J Pharmacol* **273**:113–119.

Siemiatkowski M, Maciejak P, Wislowska A, Zienowicz M, Sienkiewicz-Jarosz H, Szyndler J, *et al*. (2004). Neophobia and cortical and subcortical binding of the dopamine D2 receptor antagonist [3H]-raclopride. *Life Sci* **76**:753–761.

Siuciak J, McCarthy S, Martin A, Chapin D, Stock J, Nadeau D, *et al*. (2007). Disruption of the neurokinin-3 receptor (NK3) in mice leads to cognitive deficits. *Psychopharmacology* **194**:185–195.

Spooren W, Riemer C, Meltzer H (2005). NK3 receptor antagonists: the next generation of antipsychotics? *Nat Rev Drug Discov* **4**:967–975.

Tremblay L, Kemel ML, Desban M, Gauchy C, Glowinski J (1992). Distinct presynaptic control of dopamine release in striosomal- and matrix-enriched areas of the rat striatum by selective agonists of NK1, NK2, and NK3 tachykinin receptors. *Proc Natl Acad Sci U S A* **89**:11214–11218.

Vanderschuren LJ, Kalivas PW (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology (Berl)* **151**:99–120.

Whitty CJ, Walker PD, Goebel DJ, Poosch MS, Bannon MJ (1995). Quantitation, cellular localization and regulation of neurokinin receptor gene expression within the rat substantia nigra. *Neuroscience* **64**:419–425.

Whitty C, Paul M, Bannon M (1997). Neurokinin receptor mRNA localization in human midbrain dopamine neurons. *J Comp Neurol* **382**:394–400.

Wong JY, Clifford JJ, Massalas JS, Finkelstein DI, Horne MK, Waddington JL, Drago J (2003). Neurochemical changes in dopamine D1, D3 and D1/D3 receptor knockout mice. *Eur J Pharmacol* **472**:39–47.