

Adaptations in pre- and postsynaptic 5-HT_{1A} receptor function and cocaine supersensitivity in serotonin transporter knockout rats

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Abstract

Rationale While individual differences in vulnerability to psychostimulants have been largely attributed to dopaminergic neurotransmission, the role of serotonin is not fully understood.

Objectives To study the rewarding and motivational properties of cocaine in the serotonin transporter knockout (SERT^{-/-}) rat and the involvement of compensatory changes in 5-HT_{1A} receptor function are the objectives of the study.

Materials and methods The SERT^{-/-} rat was tested for cocaine-induced locomotor activity, cocaine-induced conditioned place preference, and intravenous cocaine self-administration. In addition, the function and expression of 5-HT_{1A} receptors was assessed using telemetry and

autoradiography, respectively, and the effect of 5-HT_{1A} receptor ligands on cocaine's psychomotor effects were studied.

Results Cocaine-induced hyperactivity and conditioned place preference, as well as intravenous cocaine self-administration were enhanced in SERT^{-/-} rats. Furthermore, SERT^{-/-} rats displayed a reduced hypothermic response to the 5-HT_{1A} receptor agonist 8-OHDPAT. S-15535, a selective somatodendritic 5-HT_{1A} receptor agonist, reduced stress-induced hyperthermia (SIH) in wild-type controls (SERT^{+/+}), while it increased SIH in SERT^{-/-} rats. As 5-HT_{1A} receptor binding was reduced in selective brain regions, these thermal responses may be indicative for desensitized 5-HT_{1A} receptors. We further found that both 8-OHDPAT and S-15535 pretreatment increased low-dose

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cocaine-induced locomotor activity in SERT^{-/-} rats, but not SERT^{+/+} rats. At a high cocaine dose, only SERT^{+/+} animals responded to 8-OHDPAT and S-15535.

Conclusion These data indicate that SERT^{-/-}-associated 5-HT_{1A} receptor adaptations facilitate low-dose cocaine effects and attenuate high-dose cocaine effects in cocaine supersensitive animals. The role of postsynaptic and somatodendritic 5-HT_{1A} receptors is discussed.

Keywords Knockout rat · Serotonin transporter · Cocaine self-administration · 5-HT_{1A} receptor · Postsynaptic · Somatodendritic

Abbreviations

CPP	conditioned place preference
DA	dopamine
DAT	dopamine transporter
ENU	N-ethyl-nitrosurea
FR	fixed ratio
NA	noradrenaline
NET	noradrenaline transporter
PR	progressive ratio
SERT	serotonin transporter
SERT ^{-/-}	homozygous serotonin transporter knockout rat
SERT ^{+/-}	heterozygous serotonin transporter knockout rat
SERT ^{+/+}	wild-type control rat
SIH	stress-induced hyperthermia
SSRI	selective serotonin reuptake inhibitor
5-HT	serotonin
5-HTTLPR	serotonin transporter-linked polymorphic region

Introduction

Drug addiction is an important health problem and a major socioeconomic issue that affects millions of people in the Western society (Uhl and Grow 2004). As the impact of cocaine addiction on health and economy escalates, so too does the need to understand its pathogenesis. Cocaine exerts its behavioral effects primarily via inhibition of the dopamine transporter (DAT), noradrenaline transporter (NET), and the serotonin transporter (SERT; Heikkila et al. 1975), with approximately equal affinity for each transporter (Reith et al. 1980). While dopamine (DA) has been the primary focus in research on individual differences in the effects of cocaine (Deminere et al. 1989), accumulating evidence suggest that serotonin (5-hydroxytryptamine, 5-HT) plays an important modulatory role in cocaine reward, although this process is not fully understood. For example, chronic treatment with the selective serotonin

reuptake inhibitor (SSRI) fluoxetine in preclinical and clinical trials resulted in both positive and negative effects on cocaine self-administration and cocaine's subjective effects (Walsh and Cunningham 1997).

The Na⁺/Cl⁻-dependent SERT regulates the re-uptake of extracellular 5-HT (Murphy et al. 1998) and thereby is the most critical regulator of extracellular 5-HT levels. Hence, SERT blockade by cocaine results into constitutive increased extracellular 5-HT levels, and secondary, activation of various 5-HT receptors that directly, or indirectly via the dopaminergic or noradrenergic systems, may influence the rewarding and motivational properties of cocaine (Acosta et al. 2005; Burmeister et al. 2004). Individual differences in central 5-HT levels have been proposed to influence personality in a trait-like manner (Serretti et al. 2006), suggesting that such individual differences are constitutive (Highley and Linnoila 1997) and genetically determined. The well-known polymorphism in the human SERT gene (5-HTTLPR; Heils et al. 1996) might underlie these individual differences, but the linkage between this polymorphism and cocaine addiction has been rarely explored, and findings are inconsistent (Mannelli et al. 2006; Gerra et al. 2007). To address how constitutive changes in central serotonergic activity and associated compensatory adaptations affect the behavioral effects of cocaine, a SERT knockout model with a disturbed serotonergic homeostasis and potential compensatory adaptations in the serotonergic system is a useful tool. A SERT knockout mouse is available (Bengel et al. 1998) and has been shown to exhibit enhanced cocaine-induced conditioned place preference (CPP; Sora et al. 1998, 2001), but reduced cocaine-induced hyperactivity (Wichems et al. 1998). Hence, it is not known whether the role of 5-HT is limited to cocaine's rewarding and psychomotor effects, or extends to cocaine's reinforcing and motivational properties that sustain intravenous cocaine self-administration behavior. The latter is of particular interest because of the high face and predictive validity with human drug self-administration behaviour (Griffith et al. 1980).

Recently, we identified a rat knockout for the SERT gene using an ENU-driven target-selected mutagenesis approach (Smits et al. 2006). Because intravenous drug self-administration has been well established in the rat, and because there is a wealth of literature available on drug-related pharmacology in this species, we used this unique rat knockout strain to address the questions whether the constitutive absence of the SERT affects cocaine's psychomotor effects, cocaine-induced conditioned place preference, and intravenous cocaine self-administration and whether compensatory adaptations are involved in the knockout's response to cocaine. The SERT knockout rat completely lacks functional SERT, displays increased extracellular 5-HT levels and a reduction in 5-HT turnover and recycling, while the presynaptic functioning of non-

serotonergic systems under basal conditions is not affected (Homberg et al. 2007). Here, we studied cocaine's psychomotor effects and intravenous cocaine self-administration in this model, and we addressed the contribution of compensatory adaptations at the 5-HT receptor level.

Material and methods

Animals

All experiments were approved by the Animal Care Committee of the Royal Dutch Academy of Science, the Free University of Amsterdam, the University Medical Center Utrecht, and the University of Groningen.

The SERT knockout ($SERT^{-/-}$) rat ($Slc6a4^{1Hubr}$) was generated by target-selected ENU-induced mutagenesis in a Wistar (Wistar/Crl) background (for detailed description, see Smits et al. 2006). Experimental animals were generated from incrosses between heterozygous SERT knockout ($SERT^{+/-}$) rats that have been outcrossed for at least six generations, and we always compared $SERT^{-/-/+/+}$ littermates. At the age of 3 weeks, ear cuts were taken under anesthesia and used for genotyping (see Smits et al. 2006 for a detailed description; Forward primer: position 3401, TCACAAAGCACTGAGACCAG; Reverse primer: position 4071, AACCTGCCAAGAGAGAGTTG). Animals were housed under standard conditions in groups of two to four per cage under controlled experimental conditions (12-h light-dark cycle, $21 \pm 1^\circ\text{C}$, 60% relative humidity, food and water ad libitum), except where stated otherwise. Only male rats were used.

Quantitative autoradiography

The procedure as described in Homberg et al. (2007) was used with minor modifications. The tissue sections were pre-incubated for 20 min at room temperature in 50 mM Tris-HCL buffer at pH 7.4 ($[^3\text{H}]$ WAY-100635). Subsequently, the sections were incubated in fresh buffer containing 3 nM $[^3\text{H}]$ WAY-100635 (40.0 Ci/mmol) for 1 h at room temperature. The air-dried sections together with $[^3\text{H}]$ MicroscalesTM standards (GE Healthcare, UK) were apposed to a $[^3\text{H}]$ hyperfilm (GE Healthcare, UK) and developed after 8 weeks. Optical densities were converted into fmol/mg of tissue equivalent using the standard curve. Nonspecific binding was determined by adding 10 μM 8-OHDPAT to the incubation buffer.

Pharmacological challenge test using body temperature telemetry

Animals were housed under a fixed 12 h light/dark period (lights off at 1 PM). Under isoflurane anesthesia, rats were

surgically equipped with a temperature-sensitive radio-transmitter (TA10TA-F40, Data Sciences, St. Paul, MN, USA) in the peritoneal cavity as described previously (Meerlo et al. 1999). After surgery, the rats were individually housed in test cages that were placed on receivers (Data Sciences, St. Paul, MN, USA) and allowed to recover for at least 1 week before drug challenge tests. Body temperature was sampled every 5 min for 10 sec starting 24 hr before the drug challenges. Drug challenge tests were performed at 4 h before lights off. Animals received saline [1 ml/kg, s.c.; stress-induced hyperthermia (SIH)], S-15535 (2.5 and 5.0 mg/kg, s.c.), and 8-OHDPAT (0.25 mg/kg, s.c.) in a randomized fashion (at least 2 days separated each challenge test).

Cocaine-induced locomotor activity

Locomotor activity was recorded by video tracking in Phenotyper[®] cages (Noldus Information Technology, Wageningen, The Netherlands; de Visser et al. 2005). The cages (45×45×45 cm), made of transparent Perspex walls and a black floor, were equipped with an empty feeding station. Each cage had a top unit containing a built-in digital infrared-sensitive video camera, infrared lighting sources, and hardware needed for video tracking (Ethovision 3.0, Noldus Information Technology, Wageningen, The Netherlands).

Male $SERT^{+/+}$ and $SERT^{-/-}$ rats were allowed to habituate to the Phenotyper cages during 30 min 1 day before the test (=novelty-induced locomotor activity). On the test day, animals underwent the following treatments: 0–30 min = habituation; 30–60 min = vehicle (saline or H₂O, i.p. or s.c.) injection; 60–80 min = pretreatment injection: saline (i.p.), 2.5 mg/kg S-15535 (s.c.), 0.25 mg/kg 8-OHDPAT (s.c.), or 0.05 and 0.3 mg/kg WAY-100635 (s.c.); 80–200 min cocaine (0, 10, 20 mg/kg, i.p.) injection. All combinations of pretreatment and cocaine dose were tested in separate naïve rats. Using Ethovision 3.0, horizontal locomotor activity was analyzed as distance moved (cm), and center activity was analyzed as time spent in the center of the cage. Data are expressed in 10 min bins.

Cocaine-induced conditioned place preference

Three-compartment Perspex boxes were used (TSE, Bad Homburg, Germany), one chamber [30×30×25 cm ($l \times w \times h$)] had black and white striped walls a wire mesh (small) floor, and one chamber [30×30×25 cm ($l \times w \times h$)] had black walls and a wire mesh (large) floor. The central space (10×30×25 cm ($l \times w \times h$)) had white walls. In both conditioning chambers and in the central space, a dim white house light (20–30 lx) was turned on. During the pretest, rats were allowed to freely explore all three chambers during 15 min. The cocaine-paired chamber was allocated in a counter-

balanced fashion to the animals' initially preferred and non-preferred chamber, respectively. During the conditioning phase, the rats were confined to a given chamber during 40 min after i.p. injection of cocaine (5, 7.5 or 10 mg/kg) or saline. On alternating days, animals received four cocaine pairings and four saline pairings in separate chambers. A 15-min test was performed 24 h after the last conditioning session, during which the animals were allowed to freely explore all compartments in a drug-free state.

Intravenous cocaine self-administration

One week before the start of the experiment, rats were equipped with intravenous silicon catheters in the right jugular vein under isoflurane anesthesia (De Vries et al. 1998). Catheter patency was maintained by daily infusion of 0.15 ml of a sterile saline solution containing heparin (20 U/ml) and weekly checked by short-lasting anesthesia induced by thiopental infused via the catheter. After surgery, rats were individually housed. The standard self-administration boxes (Med Associates, GA, USA), housed in ventilated enclosures, were fitted with two small nose-poke holes. A nose poke in the hole designated as "active" resulted in the delivery of 50 μ l of a cocaine solution in 2 s. A red house light was turned on during the entire self-administration session, a red stimulus light above the active hole was turned off when an active response was made, and remained off during the time-out period (15 s), and a yellow stimulus light in the active hole was switched on during the cocaine infusion (2 s). Nose pokes in the inactive hole had no programmed consequences. Acquisition was performed on a fixed ratio 1 (FR1) schedule during daily sessions, and cocaine dose (from 0.3 to 0.6 mg/kg/infusion) was increased when stable responding was achieved. There was no limit to the number of cocaine infusions that could be earned. During the subsequent progressive ratio (PR) schedule, the animals had to successively increase the number of nose pokes to obtain a cocaine injection according to the following sequence of required nose pokes: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc. PR sessions lasted 4 h or were terminated when 1 h elapsed without a drug infusion. Cocaine doses (0.3, 0.6 and 0.9 mg/kg/infusion) were gradually increased when stable responding was achieved. After the PR schedule the animals underwent three FR1 sessions and were subsequently subjected to 15 extinction sessions lasting 1 h during which active hole nose poking had no programmed consequences (no cocaine and no cue light).

Radioligand and drugs

4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine methanesulfonate (S-15535) was a gift of Servier Pharmaceuticals,

France. (\pm)-8-hydroxy-2-(di-*n*-propyl-amino)tetralin hydrobromide (8-OHDPAT), WAY-100635, and 5-HT were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). [3 H]WAY-100635 and [3 H]GR125743 were obtained from GE Healthcare (UK), and cocaine was purchased from O.P.G. (Utrecht, The Netherlands). S-15535, 8-OHDPAT and WAY-100635 were dissolved in H₂O, and cocaine and CP94,253 were dissolved in saline. All drug solutions were freshly prepared 1 h before the experiment and administered in a volume of 1 ml/kg.

Statistics

All data were checked for normality and homogeneity. The number of active hole pokes during acquisition of cocaine self-administration data was analyzed using two-way repeated measures ANOVA. During the PR schedule, the mean of the last two (out of four) sessions of each cocaine dose was used for two-way repeated measures ANOVA across all doses. Extinction data were divided into three parts of five sessions, and the means over the five sessions were subjected to a two-way repeated measures ANOVA. Furthermore, a *paired* Student's *t* test was used to analyze the CPP data, which were expressed as the time spent in the cocaine-paired chamber during the posttest minus the time spent in the cocaine-paired chamber during the pretest. Cocaine-induced hyperactivity was analyzed in 10 min bins and subjected to two-way ANOVA over 1 h. The pretreatment periods before the cocaine challenges were analyzed separately. The saline pretreatment group served as control and was included in all analyses. Genotype served as between factor, and time and treatment were combined as within factor. Novelty-induced locomotor activity, summed over 30 min, was analyzed using Student's *t* test. Furthermore, two-way ANOVA was used to analyze telemetry data. Finally, autoradiography densities were analyzed per brain region and subjected to two-sample Student's *t* tests. ANOVA's were followed by Newman-Keuls post hoc test when significant ($P < 0.05$) interactions were obtained [nonsignificant (n.s.)].

Results

Enhanced rewarding properties of cocaine in SERT^{-/-} rats

We tested the rewarding and motivational properties of cocaine in three different procedures that measure distinct aspects of cocaine's behavioral effects. First, we investigated cocaine reinforcement using the intravenous cocaine self-administration procedure. Although there was no significant genotype effect over 0.3 and 0.6 mg/kg cocaine per infusion during acquisition under an FR1 schedule of

reinforcement [Fig. 1a; $F_{(2,53)}=2.92$, n.s.], repeated measures ANOVA indicated that the genotype \times dose interaction was significantly different [$F_{(24,53)}=3.57$, $P<0.05$]. According to the Newman–Keuls post hoc test, $SERT^{-/-}$ rats self-administered higher amounts of cocaine when the 0.3 mg/kg cocaine dose was available than $SERT^{+/+}$ and $SERT^{+/-}$ rats [$P<0.05$], but there were no genotype differences at

0.6 mg/kg cocaine per infusion. When the knockout rats were subsequently tested under a PR schedule of reinforcement, measuring the motivation to work for a drug injection (Hodos 1961; Richardson and Roberts 1996), no genotype differences were found [Fig. 1b; $F_{(2,77)}=2.41$, n.s.], but there was a significant genotype \times dose interaction [$F_{(4,77)}=3.41$, $P<0.05$]. Post hoc testing indicated that at 0.9 mg/kg

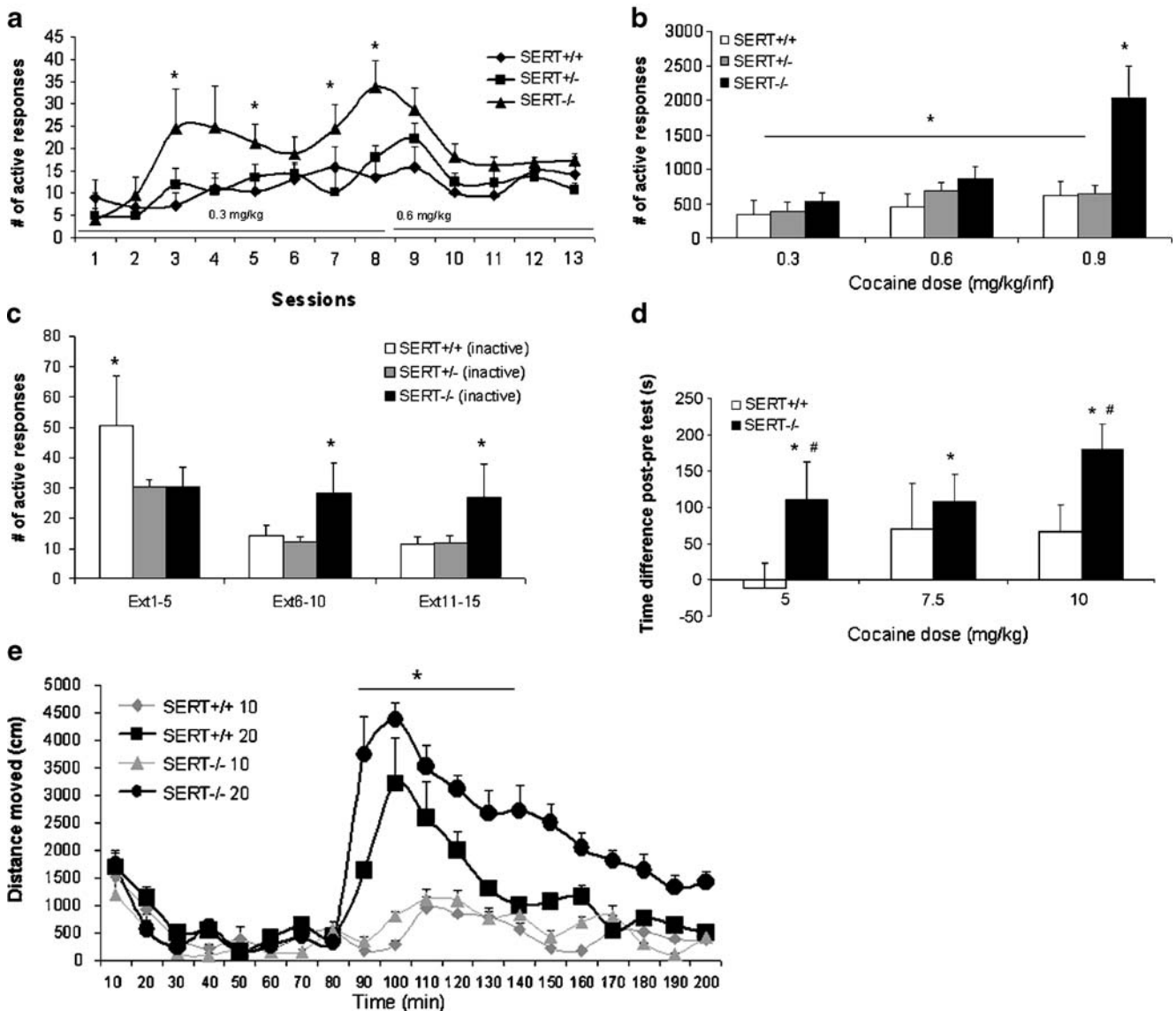


Fig. 1 **a** Acquisition of intravenous cocaine self-administration under an FR1 schedule in $SERT^{+/+}$, $SERT^{+/-}$ and $SERT^{-/-}$ rats. Male rats ($n=8-14$) had access to increasing doses of cocaine (0.3, 0.6 mg/kg/infusion) during 1-h sessions. Data represent mean (\pm SEM) number of responses made in the cocaine-paired (active) hole. * $P<0.05$ different from $SERT^{+/+}$ and $SERT^{+/-}$ rats. **b** Intravenous self-administration of increasing cocaine doses (0.3, 0.6 and 0.9 mg/kg/infusion) under a PR schedule during 4-h sessions. Data represent mean (\pm SEM) number of responses made in the cocaine-paired (active) and inactive hole. * $P<0.05$ different from $SERT^{+/+}$ and $SERT^{+/-}$ rats. **c** Extinction responding in $SERT^{+/+}$, $SERT^{+/-}$ and $SERT^{-/-}$ rats during 1-h sessions. Data represent mean (\pm SEM) number of responses made in the previously

cocaine-paired (active) hole. * $P<0.05$ different from $SERT^{+/+}$ and $SERT^{+/-}$ rats. **d** Cocaine-induced place preference induced by 5, 7.5, and 10 mg/kg cocaine in male $SERT^{+/+}$ and $SERT^{-/-}$ rats. The rats ($n=6-8$) received four cocaine and four saline conditioning trials. Time scores represent mean (\pm SEM) differences between post- and preconditioning time spent in the cocaine-paired chamber. * $P<0.05$ different from $SERT^{+/+}$ rats, # $P<0.05$ posttest different from pretest. **e** Cocaine-induced hyperactivity in male $SERT^{+/+}$ and $SERT^{-/-}$ rats ($n=4-7$). 0–30 min = habituation, 30–60 min = saline injection (i.p.), 60–80 min = saline injection (i.p.), 80–200 min = 10 or 20 mg/kg cocaine injection (i.p.). Data are expressed as mean (\pm SEM) cm distance moved in 10 min bins. * $P<0.05$ different from $SERT^{+/+}$ rats

cocaine per infusion, $SERT^{-/-}$ rats obtained significantly more cocaine infusions than $SERT^{+/+}$ and $SERT^{+/-}$ rats [$P < 0.05$], which required them to emit approximately three times more active hole responses (not shown). Inactive responding was not different during the FR1 schedule [$F_{(2,370)} = 1.35$, n.s.; not shown], but there was a significant genotype effect during the PR schedule [$F_{(2,77)} = 4.95$, $P < 0.05$]. During extinction, a significant genotype \times session interaction was found [Fig. 1c $F_{(4,77)} = 6.52$, $P < 0.0005$], attributed to higher responding in $SERT^{+/+}$ rats compared to $SERT^{-/-}$ and $SERT^{+/-}$ rats during the first five extinction sessions [$P < 0.05$] and higher responding in $SERT^{-/-}$ rats compared to $SERT^{+/+}$ and $SERT^{+/-}$ rats during sessions 6–15 [$P < 0.05$].

Next, $SERT^{+/+}$ and $SERT^{-/-}$ rats were tested in the CPP test that assesses the attribution of incentive salience to environmental stimuli associated with the subjective effects of drugs (Carr et al. 1989, Tzschentke 1998). Because the cocaine self-administration experiment did not reveal a heterozygous genotype effect, $SERT^{+/-}$ rats were not included. We found that 5 mg/kg cocaine [Fig. 1d; $T_{(1,13)}$ (genotype) = 1.8667, $P < 0.05$; $T_{(1,14)}$ ($SERT^{-/-}$, pre- vs. posttest) = 1.912, $P < 0.05$; $T_{(1,12)}$ ($SERT^{+/+}$, pre vs. posttest) = 0.3121, n.s.] and 7.5 mg/kg cocaine [$T_{(1,15)}$ (genotype) = 0.538, n.s.; $T_{(1,16)}$ ($SERT^{-/-}$, pre vs. post-test) = 3.00, $P < 0.005$; $T_{(1,14)}$ ($SERT^{+/+}$, pre vs. post-test) = 1.444, n.s.] induced conditioned place preference in $SERT^{-/-}$ rats, but not in $SERT^{+/+}$ rats. A dose of 10 mg/kg cocaine induced place preference in both genotypes, but the place preference was significantly enhanced in $SERT^{-/-}$ rats compared to $SERT^{+/+}$ rats [$T_{(1,14)}$ (genotype) = 2.229, $P < 0.05$; $T_{(1,14)}$ ($SERT^{-/-}$, pre- vs. posttest) = 3.806, $P < 0.001$; $T_{(1,14)}$ ($SERT^{+/+}$, pre- vs. posttest) = 2.237, $P < 0.05$]. This apparent

leftward, and possibly upward, shift in the dose–effect relationship of cocaine demonstrates that $SERT^{-/-}$ rats are more sensitive to the rewarding effects of the drug.

Finally, we measured the psychomotor stimulant effects of 10 and 20 mg/kg cocaine. Overall, $SERT^{-/-}$ rats, compared to $SERT^{+/+}$ rats, displayed significantly increased cocaine-induced locomotor activity [Fig. 1e; $F_{(1,120)} = 18.95$, $P < 0.0001$]. No genotype difference were observed after 10 mg/kg cocaine, and no genotype differences were observed in center time upon 10 and 20 mg/kg cocaine [$T_{(1,120)} = 0.25$; not shown]. Analysis of the first 30 min after placement into the test cage did not reveal genotype differences [$T_{(1,17)} = 0.32$], indicating that $SERT^{-/-}$ and $SERT^{+/+}$ rats do not differ in novelty-induced locomotor activity.

Desensitization of 5-HT_{1A} receptors

We measured the sensitivity of 5-HT_{1A} receptors by measuring 8-OHDPAT-induced hypothermia and the effect of S-15535 on stress-induced hyperthermia (SIH). Two-way ANOVA, including genotype, the effect of saline and of 0.25 mg/kg 8-OHDPAT over time, revealed a significant genotype effect on body temperature [Fig. 2; $F_{(2,1510)} = 29.32$, $P < 0.0001$] and a significant genotype \times time interaction [$F_{(142,1510)} = 2.89$, $P < 0.0001$]. Post hoc testing indicated that 8-OHDPAT significantly reduced body temperature in $SERT^{+/+}$ rats [$P < 0.05$], in $SERT^{+/-}$ rats [$P < 0.05$], and in $SERT^{-/-}$ rats [$P < 0.05$] over all time points, except the first 10 min in $SERT^{+/+}$ rats. In addition, 8-OHDPAT-induced hypothermia was less strongly, in $SERT^{-/-}$ rats compared to $SERT^{+/+}$ and $SERT^{+/-}$ rats [$P < 0.05$] from 20 to 60 min, and significantly different between

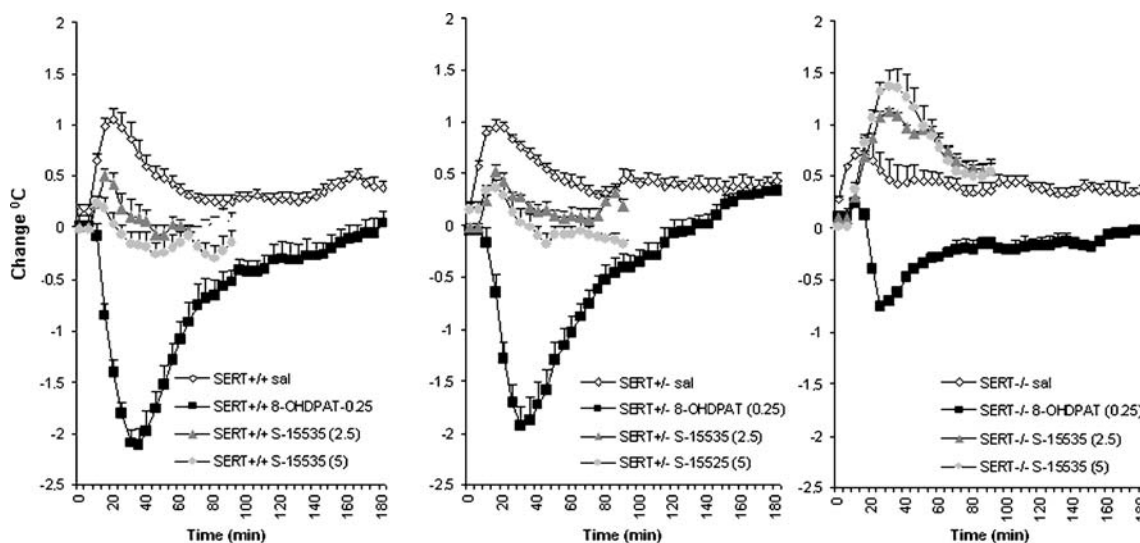


Fig. 2 a 8-OHDPAT-induced (0.25 mg/kg, s.c.) hypothermia and S-15535-mediated (2.5 and 5.0 mg/kg, s.c.) inhibition of stress-induced (handling and s.c. injection) hyperthermia in male $SERT^{+/+}$, $SERT^{+/-}$, and $SERT^{-/-}$ rats ($n = 7$). The hyperthermic response was registered for 180 min

after saline or 8-OHDPAT administration and for 90 min after S-15535 administration. Data are expressed as mean (\pm SEM) change in body temperature ($^{\circ}$ C) per 5 min. For clarity reasons, significant differences are not indicated in the figure but are described in the results section

SERT^{+/-} and SERT^{+/+} rats at 25, 35, 40, 50, and 60 min after treatment.

Analysis of the effect of 2.5 and 5.0 mg/kg S-15535 on SIH revealed a significant genotype effect [Fig. 2; $F_{(2,1119)}=113.34$, $P<0.0001$] as well as significant genotype \times time interaction [$F_{(106,1119)}=3.88$, $P<0.0001$]. Post hoc testing indicated that S-15535 reduced SIH in SERT^{+/+} [$P<0.05$] and SERT^{+/-} [$P<0.05$] rats at 5.0 mg/kg over the entire time course [$P<0.05$] and at 2.5 mg/kg from 0 to 70 min after S-15535 administration. In contrast, in SERT^{-/-} rats, SIH was significantly increased by 2.5 and 5.0 mg/kg S-15535 [$P<0.05$] from 25 to 60 min after S-15535 administration. The effect of 2.5 mg/kg S-15535 compared to 5 mg/kg S-15535 was not significantly different in all three genotypes, except for the last 15 min in SERT^{+/-} rats and the last 20 min in SERT^{+/+} rats. The response to both doses of S-15535 was significantly different between SERT^{-/-} rats compared to SERT^{+/-} and SERT^{+/+} rats [$P<0.05$] from 20 min to the end of the measurement, except for 2.5 mg/kg S-15535 at 80 and 85 min. Furthermore, the SIH was significantly decreased in SERT^{-/-} rats compared to SERT^{+/+} and SERT^{+/-} rats at 15–30 min after the saline injection [$P<0.05$]. Finally, no genotype differences were found between SERT^{+/+} and SERT^{+/-} rats.

[³H]WAY-100635 binding to 5-HT_{1A} receptors was reduced in the dorsal raphe nuclei, in the CA2 and CA3 regions of the hippocampus, and in the cingulate cortex of SERT^{-/-} rats. No genotype differences were found in the CA1 region of the hippocampus, the septum, and piriform cortex (Fig. 3; Table 1).

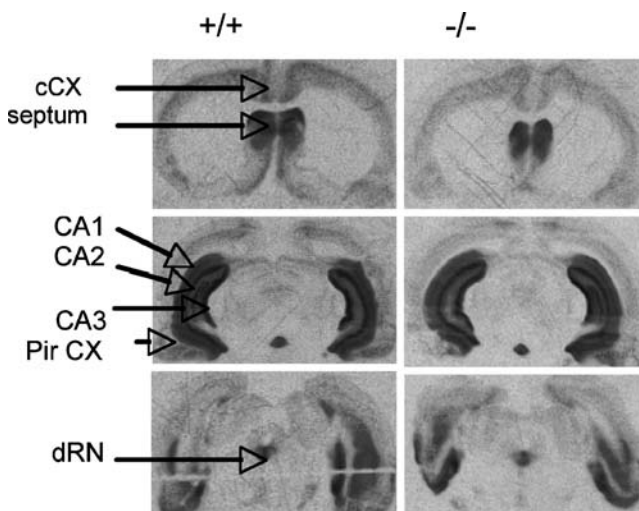


Fig. 3 Representative [³H]WAY-100635 autoradiograms of coronal sections from male SERT^{+/+} and SERT^{-/-} rats ($n=2-3$). CX Cingulate cortex, CA1, CA2, CA3 hippocampal layers, dRN dorsal raphe nucleus, pir CX piriform cortex. See Table 1 for 5-HT_{1A} receptor quantification

5-HT_{1A} receptor desensitization interferes with enhanced cocaine-induced hyperactivity in SERT^{-/-} rats

To study whether the SERT^{-/-}-mediated altered responsiveness to 5-HT_{1A} receptor agonists contributes to cocaine supersensitivity, we tested the effect of 8-OHDPAT and S15535 on cocaine's psychomotor effects induced by a low (10 mg/kg) and high (20 mg/kg) dose of the drug. 8-OHDPAT, 0.25 mg/kg, pretreatment before 10 or 20 mg/kg (Fig. 4a,b) cocaine challenges induced a significant overall pretreatment effect [$F_{(1,34)}$ (pretreatment 8-OHDPAT vs. vehicle–10 mg/kg cocaine)=13.59, $P<0.001$; $F_{(1,50)}$ (pretreatment 8-OHDPAT vs. vehicle–20 mg/kg cocaine)=46.22, $P<0.0001$]. This indicates that 8-OHDPAT induced activity more strongly in SERT^{+/+} rats compared to SERT^{-/-} rats [$P<0.05$], which was accompanied with signs of the 5-HT syndrome (flat body posture and forepaw treading; not shown) in SERT^{+/+} rats, but not SERT^{-/-} rats. A subsequent 10-mg/kg cocaine challenge induced an overall highly significant genotype effect (Fig. 4a; $F_{(1,104)}=30.79$, $P<0.0001$), which was due to an increased locomotor response in SERT^{-/-} rats. Upon a 20-mg/kg cocaine challenge, the 8-OHDPAT pretreatment did not induce an overall pretreatment effect [Fig. 4b; $F_{(1,143)}=2.65$, n.s.], but did induce a significant genotype \times pretreatment interaction [$F_{(11,143)}=4.79$, $P<0.0001$]. Post hoc testing indicated that at 10 [$P<0.05$] and 20 [$P<0.05$] min after the 20 mg/kg cocaine challenge, 8-OHDPAT pretreatment significantly enhanced locomotor activity in SERT^{+/+} rats compared to SERT^{-/-} rats. Furthermore, 8-OHDPAT pretreatment significantly enhanced locomotor activity in SERT^{+/+} rats at 10, 20, 40, 50, and 60 min after the cocaine challenge compared to vehicle pretreatment in SERT^{+/+} rats [$P<0.05$], while the locomotor response to 20 mg/kg cocaine was not further enhanced in 8-OHDPAT pretreated SERT^{-/-} rats compared to vehicle pretreated SERT^{-/-} rats. Analysis of centre time revealed that 8-OHDPAT had no significant effect compared to saline pretreatment upon the 10 mg/kg cocaine challenge [$F_{(11,104)}$ (pretreatment effect)=1.83, n.s.; $F_{(1,104)}$ (genotype effect) <0.001 , n.s.; not shown]. Upon the 20 mg/kg cocaine challenge, 8-OHDPAT decreased center time in both SERT^{+/+} and SERT^{-/-} [$F_{(11,143)}=6.61$, $P<0.0001$; not shown], without genotype differences [$F_{(1,143)}<0.001$, n.s.; not shown].

In contrast to 8-OHDPAT, S-15535 (2.5 mg/kg) pretreatment (before cocaine) did not induce an overall pretreatment effect [Fig. 4c $F_{(1,36)}$ (pretreatment S-15535 vs. vehicle–10 mg/kg cocaine)=0.59, n.s.; $F_{(1,56)}$ (pretreatment S-15535 vs. vehicle–20 mg/kg cocaine)=1.20, n.s.]. Upon the 10 mg/kg cocaine challenge, there was a highly significant overall genotype effect [Fig. 4c; $F_{(1,109)}=26.49$, $P<0.0001$] and genotype \times pretreatment interaction [$F_{(11,109)}=2.32$, $P<0.05$]. Post hoc testing revealed that at

Table 1 5-HT_{1A} receptor quantification in brain areas of SERT^{-/-} rats and control littermates

Region	[³ H]WAY-100635			Student's <i>t</i> test
	Bregma	SERT ^{+/+}	SERT ^{-/-}	
Septum	0.12	150±12.8	156±11.2	<i>T</i> _(1,17) =0.28
Cingulate cortex	0.12	72±1.8	47±1.7 ^a	<i>T</i> _(1,25) =9.46, <i>P</i> <0.0001
Piriform cortex	-4.68	78±5.6	77±5.3	<i>T</i> _(1,20) =0.045
CA1 (hippocampus)	-4.68	209±19.3	167±1.8	<i>T</i> _(1,13) =1.74
CA2 (hippocampus)	-4.68	239±19.6	190±14.1 ^a	<i>T</i> _(1,14) =2.05, <i>P</i> <0.05
CA3 (hippocampus)	-4.68	223±15.6	178±0.8 ^a	<i>T</i> _(1,12) =1.88, <i>P</i> <0.05
Dorsal RN	-6.50	107±4.2	89±0.6 ^a	<i>T</i> _(1,2) =3.88, <i>P</i> <0.05

Optical densities were converted into fmol/mg of tissue equivalent using [³H]Microscales™ standards. The data represent mean fmol/mg tissue (±SEM). ^aSERT^{-/-} different from SERT^{+/+}

10, 20, 30, 40, and 50 min after the 10 mg/kg cocaine challenge, S-15535-pretreated SERT^{-/-} rats displayed increased locomotor activity compared to SERT^{+/+} rats [*P*<0.05]. Relative to vehicle pretreatment, S-15535 pretreatment potentiated the locomotor response induced by 10 mg/kg cocaine in SERT^{+/+} rats at 10 and 20 min after the cocaine challenge [*P*<0.05] and at 10, 20, 30, 40 and 50 min after the cocaine challenge in SERT^{-/-} rats [*P*<0.05]. Upon 20 mg/kg cocaine, there was a significant overall genotype effect [Fig. 4d; *F*_(1,190)=15.96, *P*<0.0001]. The overall genotype effect was most likely caused by the increased cocaine-induced locomotor response in saline-pretreated SERT^{-/-} rats, while the locomotor responses after S-15535 were comparable in SERT^{-/-} and SERT^{+/+} rats. These data show that particularly SERT^{+/+} rats increased their responding to 20 mg/kg dose of cocaine after S-15535 pretreatment. In contrast to 8-OHDPAT, S-15535 increased center time at 20 mg/kg cocaine, in SERT^{+/+} rats only [*F*_(1,190)=4.50, *P*<0.05; not shown]. At 10 mg/kg cocaine, S-15535 pretreatment had no significant effect on center time [*F*_(11,109) (pretreatment effect)=0.51, n.s.; *F*_(11,109) (genotype effect)=0.00, n.s.; not shown].

Pretreatment with 0.3 mg/kg WAY-100635, a dose suggested to inhibit postsynaptic 5-HT_{1A} receptors (Müller et al. 2007), by itself had no genotype or pretreatment effect [Fig. 4e; *F*_(1,41)=0, n.s.]. Likewise, 0.05 mg/kg WAY-100635, a dose assumed to inhibit presynaptic 5-HT_{1A} receptors (Carey et al. 2005), did not affect locomotor activity in SERT^{+/+} and SERT^{-/-} rats [Fig. 4f; *F*_(1,46)=1.32, n.s.]. After the 20-mg/kg cocaine challenge, pretreatment with 0.3 mg/kg WAY-100635 induced a significant overall genotype [Fig. 4e; *F*_(1,134)=6.40, *P*<0.05] and genotype × pretreatment effect [*F*_(11,134)=2.57, *P*<0.01]. According to Newman-Keuls post hoc test, there were no genotype difference when 0.3 mg/kg WAY-100635 pretreated SERT^{+/+} and SERT^{-/-} rats were compared. However, 0.3 mg/kg WAY-100635 pretreatment reduced cocaine-induced hyperactivity relative to vehicle pretreatment in SERT^{-/-} rats at 10, 20, 30, 40, and 60 min after the cocaine injection [*P*<0.05], while in SERT^{+/+} rats, WAY-100635 pretreatment, compared to vehicle pretreat-

ment, significantly reduced cocaine-induced activity only at 20 min after the cocaine challenge [*P*<0.05]. A 0.05-mg/kg WAY-100635 pretreatment also induced an overall genotype effect [Fig. 4f; *F*_(1,130)=9.78, *P*<0.005], but no significant genotype × pretreatment interaction was found [*F*_(11,130)=1.15, n.s.]. Center time was significantly enhanced in SERT^{+/+} and SERT^{-/-} rats treated with 0.3 mg/kg WAY100635 and 20 mg/kg cocaine [*F*_(11,134)=19.16, *P*<0.0001; not shown], without genotype differences [*F*_(1,134)=0.18, n.s.; not shown]. A similar response pattern was observed after 0.05 mg/kg WAY100635 combined with 20 mg/kg cocaine [*F*_(11,130) (pretreatment effect)=24.75, *P*<0.0001; *F*_(1,130) (genotype effect)=1.98, n.s.; not shown].

Discussion

SERT^{-/-} rats are supersensitive to cocaine

Here, we show that SERT^{-/-} rats are supersensitive to cocaine as measured in three distinct behavioral procedures, namely, cocaine-induced locomotor activity, cocaine-induced CPP, and intravenous cocaine self-administration. SERT^{-/-} mice also display enhanced cocaine-induced CPP (Sora et al. 1998, 2001, but see Hall et al. 2002), but in contrast to the SERT^{-/-} rat, cocaine-induced hyperactivity is reduced in SERT^{-/-} mice (Wichems et al. 1998). Procedural differences may underlie these contrasting findings, but there may also be species differences regarding developmental adaptations in response to the absence of the SERT or interference with genetic background that differ between rat and mouse strains. We also show that 5-HT_{1A} receptor agonists differentially affect cocaine's psychomotor effects in SERT^{-/-} and SERT^{+/+} rats at low and high cocaine doses, implying that a mechanism through which cocaine supersensitivity is mediated in SERT^{-/-} rats involves, among other 5-HT receptors, the 5-HT_{1A} receptor. Together, three important conclusions can be drawn from this study: (1) cocaine-induced locomotor activity in SERT^{-/-} rats is predictive for enhanced intrave-

nous cocaine self-administration, implying common underlying mechanisms, (2) 5-HT plays an important modulatory role in the mechanisms by which cocaine exerts its behavioural effects, and (3) compensatory adaptations in 5-HT_{1A} receptor function may contribute to the cocaine supersensitivity in SERT^{-/-} rats.

Altered 5-HT_{1A} receptor responsivity in SERT^{-/-} rats

We previously observed a profound increase in extracellular 5-HT levels in SERT^{-/-} rats (Homberg et al. 2007) that could potentially affect the functioning of 5-HT receptors, as has been reported after chronic SSRI treatment (Blier et

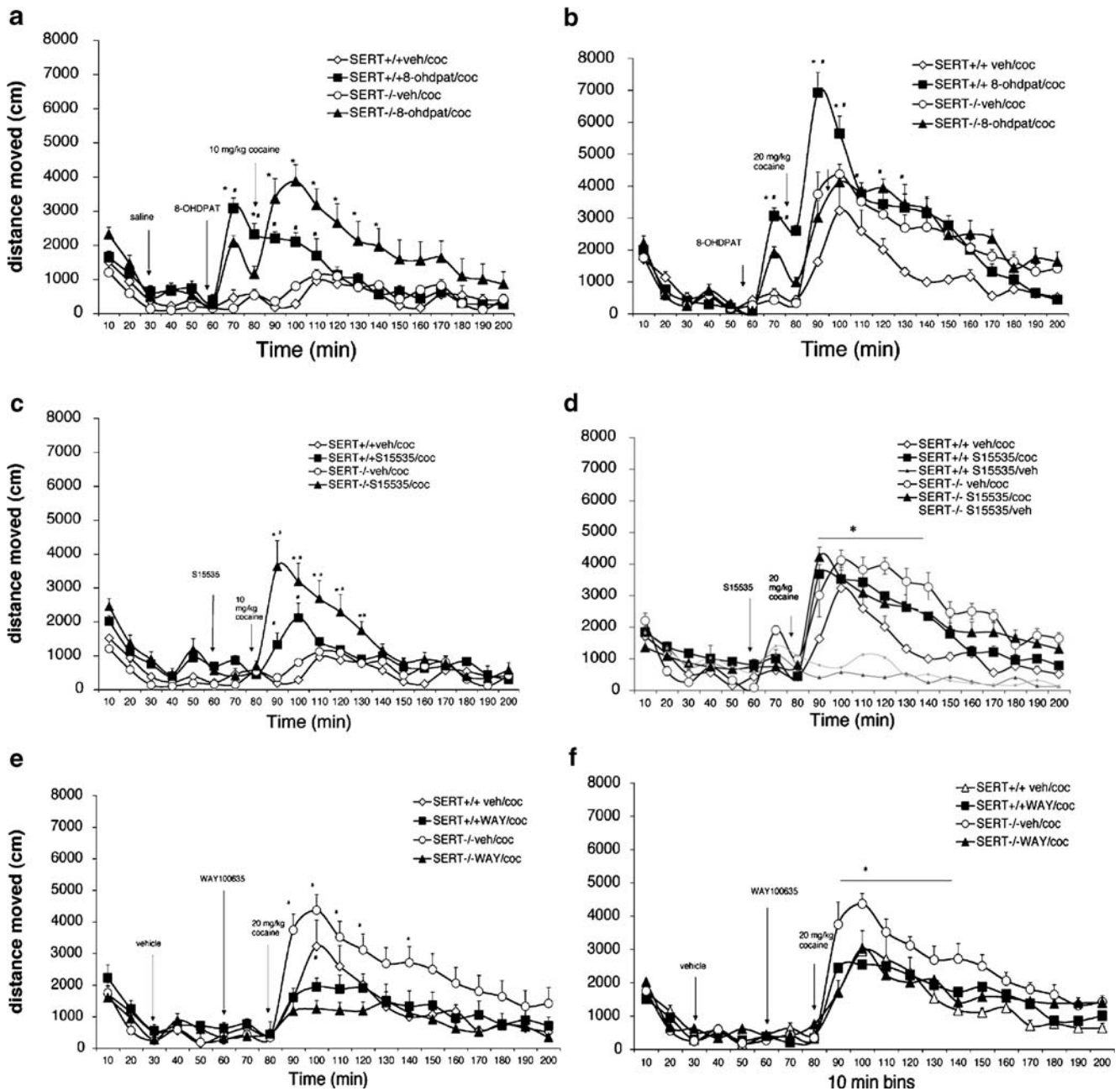


Fig. 4 Effect of 0.25 mg/kg 8-OHDPAT (s.c.) pretreatment on locomotor activity in male SERT^{+/+} and SERT^{-/-} rats induced by **a** 10 mg/kg (*n*=5) and **b** 20 mg/kg (*n*=6) cocaine. Effect of 2.5 mg/kg S-15535 (s.c.) pretreatment on locomotor activity in male SERT^{+/+} and SERT^{-/-} rats induced by **c** 10 mg/kg (*n*=5–6) and **d** 20 mg/kg (*n*=10–11) cocaine. The locomotor response to 20 mg/kg cocaine after **e** 0.3 mg/kg WAY-100635 (*n*=5) and **f** 0.05 mg/kg WAY-100635 (*n*=5) pretreatment. Habituation took place at 0–30 min; from 30 to 60 min, the effect of a

vehicle injection (s.c.) was measured; at 60 min after start of the test the animals received 8-OHDPAT, S-15535 or WAY100635 pretreatment (s.c.); and 20 min later (80 min after start of the test), the animals received a 10- or 20-mg/kg cocaine challenge (i.p.). Data represent mean (±SEM) distance moved (cm) in 10 min bins. **P*<0.05 SERT^{+/+} vs. SERT^{-/-} rats; #*P*<0.05 SERT^{+/+} vehicle vs. SERT^{+/+} pretreatment, ^a*P*<0.05 SERT^{-/-} vehicle vs. SERT^{-/-} pretreatment

al. 1990) and in human carrying the s/s genotype of the 5-HTTLPR (David et al. 2005). We found that 8-OHDPAT-induced hypothermia was decreased in $SERT^{-/-}$ rats and that S15535-induced attenuation of SIH as found in $SERT^{+/+}$ and $SERT^{+/-}$ rats was converted into a potentiation of the SIH in $SERT^{-/-}$ rats. These findings may either be explained by constitutive occupation of 5-HT_{1A} receptors because of the high endogenous 5-HT tonus or by desensitization of the receptors as a compensatory adaptive response to the high endogenous 5-HT tonus, or both. Autoradiography revealed significant decreases in 5-HT_{1A} receptor density in the raphe nuclei and several terminal regions, suggesting that 5-HT_{1A} receptors are (slightly) desensitized in $SERT^{-/-}$ rats. To a large extent, corresponding findings have been obtained in $SERT^{-/-}$ mice (e.g., Li et al. 1999; Fabre et al. 2000). Nevertheless, it is still possible that the reduced 5-HT_{1A} receptor-mediated hypothermic responses in $SERT^{-/-}$ rats (partially) reflects occupation of (agonist-desensitized) 5-HT_{1A} receptors because of the high endogenous 5-HT tonus.

In rats (e.g., Millan et al. 1993b; Bill et al. 1991; Goodwin et al. 1987) and humans (Blier et al. 2002), 5-HT_{1A} receptor-induced hypothermia is thought to be mediated by postsynaptic 5-HT_{1A} receptors, while in mice, the somatodendritic 5-HT_{1A} receptors seem to be involved (Goodwin et al. 1985; Martin et al. 1992), although some controversies regarding the pre- and postsynaptic modulation of 5-HT_{1A} receptor-mediated hypothermia in rat exist (Higgins et al. 1988; Hillegaart 1991). However, combined with our observation that 8-OHDPAT-induced activity and 5-HT syndrome, which are postsynaptically mediated (Yamada et al. 1988), were reduced in $SERT^{-/-}$ rats relative to $SERT^{+/+}$ rats in the locomotor tests, the 8-OHDPAT-induced hypothermia findings imply that predominantly postsynaptic 5-HT_{1A} receptors are involved. This view is further supported by the lack of a hypothermic effect of S-15535 (Millan et al. 1993a; de Boer et al. 2000). Previous studies have convincingly demonstrated that this compound is a full somatodendritic 5-HT_{1A} receptor agonist and has only weak partial agonistic or even antagonistic properties at postsynaptic 5-HT_{1A} receptor (Millan et al. 1993a; Newman-Tancredi et al. 1999; de Boer et al. 2000).

Regarding the SIH, it is not clear whether this short-lasting thermogenic effect is mediated by somatodendritic or postsynaptic 5-HT_{1A} receptors, or both. The fact that S-15535 dose-dependently attenuated SIH in $SERT^{+/+}$ and $SERT^{+/-}$ rats indicates involvement of somatodendritic sites. Strikingly, S-15535 enhanced rather than reduced SIH in $SERT^{-/-}$ rats. Considering the high 5-HT tonus in $SERT^{-/-}$ rats, and thus increased occupation of 5-HT_{1A} receptors, this most probably reflects the antagonistic properties of S-15535 at (agonist-desensitized) postsynaptic 5-HT_{1A} receptors. In support, WAY-100635, the prototyp-

ical and selective 5-HT_{1A} receptor antagonist, similarly enhances SIH in $SERT^{-/-}$ rats only (unpublished observations, J.O. and S.d.B.). In the $SERT^{-/-}$ rats, this antagonistic effect may have masked the somatodendritic effects of S-15535, and therefore, the S15535-SIH data do not allow us to conclude as to whether the function of somatodendritic 5-HT_{1A} receptors has been changed. The pharmacological profile of S-15535, combined with the reduced 5-HT_{1A} receptor binding in the raphe nuclei of $SERT^{-/-}$ rats, and the finding that somatodendritic 5-HT_{1A} receptors are strongly desensitized in $SERT^{-/-}$ mice (Gobbi et al. 2001), show that the S-15535-SIH data may point to desensitized somatodendritic 5-HT_{1A} receptors in $SERT^{-/-}$ rats.

Altered 5-HT_{1A} receptor responsivity contributes to cocaine supersensitivity

The effect of cocaine on extracellular monoamine levels is dependent upon the activity of the monoamine transporters. Because the presynaptic functioning of non-serotonergic neurons is not affected in the $SERT^{-/-}$ rat, at least under basal conditions (Homberg et al. 2007), and a differential monoamine response to a cocaine challenge is only seen for 5-HT in $SERT^{-/-}$ mice and rats (Shen et al. 2004; J.H., E.C., J.O., unpublished observations), our results imply that the observed cocaine supersensitivity is not exerted by noradrenergic and dopaminergic systems, but results from a primary effect of the disturbed serotonergic system. However, in view of the complex interactions between central 5-HT, DA and NA systems, we cannot exclude that alterations in mesolimbic dopaminergic or noradrenergic receptor density or sensitivity contribute to the behavioral changes in $SERT^{-/-}$ rats.

The current idea concerning the role of 5-HT in the behavioural effects of cocaine involves an inhibitory role of 5-HT. Thus, increasing 5-HT levels generally inhibits cocaine self-administration (Carroll et al. 1990; Richardson and Roberts 1991; Peltier and Schenk 1993; but see Porrino et al. 1989; Tella 1995). Because cocaine-induced 5-HT release is reduced in $SERT^{-/-}$ mice and rats (Shen et al. 2004; J.H., E.C., J.O., unpublished observations), because both $SERT^{-/-}$ mice and rats show increased cocaine-induced CPP (Sora et al. 1998, 2001; present study), and because novelty-induced locomotor activity was comparable in $SERT^{+/+}$ and $SERT^{-/-}$ rats, cocaine supersensitivity in $SERT^{-/-}$ models may be explained by reduced 5-HT dampening of the cocaine's effects. The mechanism underlying this 5-HT dampening is not understood. As activation of inhibitory somatodendritic 5-HT_{1A} receptors reduces the firing rate of raphe neurons and subsequently 5-HT and DA release in terminal regions (Yoshimoto and McBride 1992; Herges and Taylor 1999; Andrews et al. 2005), desensitization of somatodendritic 5-HT_{1A} receptors in $SERT^{-/-}$ models may mediate the reduced 5-HT

dampening and cocaine supersensitivity. On the other hand, there are also indications that somatodendritic 5-HT_{1A} receptors facilitate psychostimulant addiction-related behaviors and that postsynaptic 5-HT_{1A} receptors predominantly inhibit psychostimulant-induced locomotor activity (Müller et al. 2007). In this respect, desensitization of postsynaptic 5-HT_{1A} receptors in SERT^{-/-} models may potentiate cocaine's psychomotor effects, and desensitization of somatodendritic 5-HT_{1A} receptors may exert an inhibitory effect. To extend the thermal findings and to study whether and how 5-HT_{1A} receptors contribute to SERT^{-/-}-induced cocaine supersensitivity, we tested the effects of 5-HT_{1A} receptor ligands on cocaine's psychomotor effects. Cocaine-induced hyperactivity was chosen as behavioural indicator because it may have predictive value for intravenous cocaine self-administration in SERT^{-/-} rats and may be more readily to interpret. Both S-15535 and 8-OHDPAT were found to enhance cocaine's psychomotor effects in SERT^{+/+} and SERT^{-/-} rats. While the effects of 0.25 mg/kg 8-OHDPAT are most likely to be mediated by postsynaptic receptors, as reported by others (Müller et al. 2007), the effects of S-15535 may be more difficult to interpret because of its pharmacological profile (Millan et al. 1993a; Newman-Tancredi et al. 1999; de Boer et al. 2000). In contrast to 8-OHDPAT, S-15535 by itself had no effect on activity, which suggests at least that S-15535 did not act as postsynaptic 5-HT_{1A} receptor agonist. A postsynaptic antagonistic effect is also not likely, as both S-15535 and 8-OHDPAT enhanced cocaine's psychomotor effects. Activation of somatodendritic 5-HT_{1A} receptors is known to be associated with a reduction in spontaneous activity (Müller et al. 2007), and thus the most likely explanation is that the failure of S-15535 to affect locomotor activity in habituated animals reflects a somatodendritic agonistic effect. If this assumption is correct, our data show that activation of both somatodendritic and postsynaptic 5-HT_{1A} receptors facilitate cocaine's effects in SERT^{-/-} rats. Furthermore, because SERT^{-/-} rats were highly responsive to 8-OHDPAT and S-15535, despite their high endogenous 5-HT tonus, it is most likely that desensitization of postsynaptic and somatodendritic 5-HT_{1A} receptors in the SERT^{-/-} rat facilitates the effects of low doses of cocaine, rather than receptor occupation.

At a high cocaine dose (20 mg/kg), 8-OHDPAT and S-15535 pretreatment had no further effects in SERT^{-/-} rats, possibly because somatodendritic and postsynaptic 5-HT_{1A} receptors in SERT^{-/-} were maximally activated. The selective 5-HT_{1A} receptor antagonist WAY-100635, at doses inhibiting either somatodendritic (0.05 mg/kg) or postsynaptic 5-HT_{1A} receptors (0.3 mg/kg), reduces cocaine-induced hyperactivity in SERT^{-/-}, but not SERT^{+/+} rats, which supports this suggestion. Thus, there could have been more competition between 5-HT and WAY-100635 to bind to the 5-HT_{1A} receptor in SERT^{-/-} rats. As cocaine

more effectively induced 5-HT release in SERT^{+/+} mice/rats compared to SERT^{-/-} mice (Shen et al. 2004) and rats (J.H., E.C., J.O., unpublished observations), receptor occupation by endogenous 5-HT does not seem to explain these findings. It is more likely that the reduced responsiveness to 8-OHDPAT and S-15535 at high cocaine doses is also due to 5-HT_{1A} receptor desensitization. Hence, the remarkable finding is that 5-HT_{1A} receptor desensitization facilitates low-dose cocaine effects, while attenuating high dose cocaine effects. This implies that genotype differences between SERT^{-/-} and SERT^{+/+} rats in cocaine sensitivity will mainly be found at low cocaine doses, as was seen during FR1 responding in the self-administration procedure. Yet, in the present study, cocaine doses may not have been high enough to diminish genotype differences in the locomotor and CPP tests.

The effects of 8-OHDPAT and S-15535 differed in that 8-OHDPAT combined with 20 mg/kg cocaine affected the intensity of the locomotor response in SERT^{+/+} rats, while S-15535 combined with the same dose of cocaine reduced the response latency and increased the duration of the locomotor response in these animals. These findings argue against the possibility that reduced cocaine-induced locomotor activity in SERT^{+/+} rats after saline pretreatment reflected stereotypy. Although speculative, it might be that the response intensity is mediated by 5-HT_{1A} receptors in mesolimbic structures that are known to play a central role in reward (Koob 1992) and that somatodendritic 5-HT_{1A} receptors affect the duration of the response by their control over raphe firing rate and thus 5-HT release and synthesis throughout the brain. Our data suggest that the latter mechanism is sated earlier than the postsynaptic mechanism regarding cocaine-induced locomotor activity.

An increase in center time in an open field test is generally interpreted as an anxiolytic effect. Because center time did not differ between saline-pretreated SERT^{+/+} and SERT^{-/-} rats, it is not likely that genotype differences in sensitivity to cocaine's anxiolytic effects explain the increased sensitivity to cocaine-induced locomotor activity in SERT^{-/-} rats. Strikingly, while 8-OHDPAT combined with 20 mg/kg cocaine reduced center time, S-15535 increased center time, in SERT^{+/+} rats only. Consistent with the present observation, De la Garza and Cunningham (2000) reported that 0.2 mg/kg 8-OHDPAT increased peripheral locomotor activity, while it reduced activity in the center of the open field. The increase in center time after S-15535 pretreatment implies that, while 8-OHDPAT mediates an anxiogenic effect combined with cocaine, S-15535 has an anxiolytic effect. Because both 8-OHDPAT and S-15535 have anxiolytic effects in classical tests of anxiety (Dekeyne et al. 2000) and because center time does not seem to be proportional to the horizontal locomotor response, anxiety cannot explain the present observations.

8-OHDPAT decreases (Müller et al. 2003) and WAY-100635 increases (Müller et al. 2002) cocaine-induced 5-HT release in the nucleus accumbens. Furthermore, S-15535 suppresses dialysate levels of 5-HT in the hippocampus (Dekeyne et al. 2000). It is therefore tempting to speculate that S-15535 and 8-OHDPAT increased cocaine's psychomotor effects by shifting the 5-HT-DA balance to the more dopaminergic side when 5-HT levels decrease (Carey et al. 2004, 2005). In SERT^{-/-} models, relatively reduced cocaine-induced 5-HT release (Shen et al. 2004) could thus result in a more dopaminergic type of response to cocaine. Because cocaine-induced dopamine, and also noradrenaline, release is not changed in the prefrontal cortex, caudate putamen, nucleus accumbens, and hippocampus of SERT^{-/-} models (Shen et al. 2004; J.H., E.C., J.O., unpublished observations), such a serotonin to dopamine shift should be an indirect one. It could be that indirect pathways via desensitized 5-HT_{1A} receptors are involved in SERT^{-/-} rats. In addition, other 5-HT receptors may contribute to such indirect pathways, as we found that the 5-HT_{1B} receptor agonist CP94,253 also influenced cocaine-induced locomotor activity differentially in SERT^{-/-} and SERT^{+/+} rats, respectively (J.H. unpublished observations). Identification of compensatory mechanisms affecting cocaine supersensitivity in SERT^{-/-} rats may ultimately contribute to the development of more effective treatments for cocaine vulnerable subjects.

Interestingly, in the cocaine self-administration procedure, responding was enhanced during the FR1 schedule (acquisition), when a low dose of cocaine was available, and during the PR schedule, when a high dose of cocaine was available. Thus, the genotype effect was schedule dependent, in such a way that an effect was only seen when the cocaine/response ratio was low. This suggests that SERT^{-/-} rats were prepared to work harder to obtain reward and that it is not likely that increased responding is because stereotypy. Similarly, fluoxetine and 8-OHDPAT only decreased cocaine self-administration when responding was high (Peltier and Schenk 1993), implying that the desensitized postsynaptic 5-HT_{1A} receptors in SERT^{-/-} rats are involved in "cost-to-benefit" scaling. Furthermore, during the PR schedule, but not the FR1 schedule, the number of inactive responses was significantly increased in SERT^{-/-} rats. Hence, the increased PR responding in these animals may relate to insensitivity to extinction. During extinction, responding was increased in SERT^{+/+} compared to SERT^{+/-} and SERT^{-/-} rats across sessions 1–5, but during subsequent sessions, SERT^{-/-} rats failed to extinguish responding towards the previously rewarded nose poke hole. The reduced cocaine/response ratio in SERT^{-/-}, at least during the PR schedule, might therefore reflect perseveration. The finding that central 5-HT depletion (Tran-Nguyen et al. 2001) reduces extinction responding in cocaine-trained animals is in line with the

present observation. Paradoxically, chronic fluoxetine treatment in cocaine-trained adult rats reduces extinction responding (Baker et al. 2001), while prenatal chronic fluoxetine exposure increases extinction responding and, in addition, increases cocaine-induced conditioned place preference (Forcelli and Heinrichs 2008). Considering 5-HT as a potent neurotropic factor (Lauder 1990), early excess 5-HT, induced by prenatal fluoxetine or genetic inactivation of the serotonin transporter, may cause persistent neuroadaptations. Although highly speculative, these neuroadaptations may involve those found in relation to the 5-HTTLPR; a functional uncoupling between the anterior cingulate cortex and amygdala is hypothesized to be associated with emotional perseveration (Pezawas et al. 2005).

Conclusion

Taken together, we show that SERT^{-/-} rats are supersensitive to cocaine in three behavioural tests measuring distinct aspects of cocaine's effects. Importantly, this behavioral phenotype is not only attributable to the absence of the SERT, but is also influenced by desensitization of 5-HT_{1A} receptors and possibly by functional changes in other 5-HT receptors. Our data not only highlight the role of 5-HT_{1A} receptors in cocaine vulnerability, but also stress the importance of addressing compensatory adaptations in SERT^{-/-} and other knockout models, as they are relevant to constitutive trait-like individual differences in central 5-HT levels which may require individualized pharmacotherapy. For these reasons, future studies should extend our findings to the intravenous drug self-administration procedure.

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