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Serotonin transporter deficiency in rats improves inhibitory control but not behavioural flexibility

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Keywords: aggression, 5-CSRTT, 5-HIAA, knockout, reversal learning

Abstract
Impulsivity and aggression have been suggested to inversely correlate with central serotonin (5-HT) levels in a trait-like manner. However, this relationship is far from straightforward. In the present study we addressed the effect of lifelong reduced or absent serotonin transporter (SERT) function, which is associated with constitutively increased extracellular 5-HT levels, on impulsivity and aggression. We used unique SERT knockout rats in a resident–intruder test, five-choice serial reaction time task and serial reversal learning task to assay aggression, inhibitory control and behavioural flexibility, respectively. Homozygous SERT knockout rats (SERT⁻⁻) displayed reduced aggression and improved inhibitory control, but unchanged behavioural flexibility. The behavioural phenotype of heterozygous SERT knockout rats (SERT⁺⁻) was not different from that of wild-type controls in any of the behavioural paradigms. We determined monoamine (metabolite) tissue levels in the medial prefrontal cortex, orbitofrontal cortex, lateral hypothalamus, raphe nuclei and cerebrospinal fluid, and found that the 5-HT levels, but not other monoamine tissue levels, were reduced in SERT⁻⁻ rats. In addition, the 5-hydroxyindoleacetic acid (5-HIAA)/5-HT ratio in cerebrospinal fluid was increased in these rats. In conclusion, our data show that the absence of the SERT affects aggression and inhibitory control, but not behavioural flexibility, characteristics that may reflect the trait-like consequences of constitutive changes in central 5-HT levels.

Introduction
The brain serotonergic system plays an important role in aggression and executive functions, such as inhibitory control processes that subserve impulsivity (Eveden, 1999; Olivier, 2004). Based on a wealth of clinical and preclinical observations (e.g. Linnoila et al., 1983; Soubrie, 1986; Vergnes et al., 1986; Coccaro & Kavoussi, 1997; Harrison et al., 1997, 1999; Fairbanks et al., 2001), the hypothesis has been posed that the activity of the central serotonergic system (5-hydroxytryptamine, 5-HT) is inversely correlated with impulsivity and aggression, possibly in a trait-like manner (Highley & Linnoila, 1997; Serretti et al., 2006). In support of this, 5-HT-releasing agents have therapeutic efficacy in both human and nonhuman aggression and other impulse control disturbances (Fuller, 1997; Brady et al., 1998; Cherek & Lane, 2000), lumbar cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) levels are reduced in impulsive subjects (Linnoila et al., 1983), and acute tryptophan depletion increases behavioural disinhibition (LeMarquand et al., 1998, 1999). Nonetheless, the relationship between central 5-HT function and impulsivity and aggression is far from straightforward, as various studies did not find an effect of indirect 5-HT manipulations on impulsivity (Clark et al., 2005; Cools et al., 2005), or found a positive correlation between 5-HT levels and impulsive behaviour (Puumala & Sirvio, 1998; Dalley et al., 2002).

Central extracellular 5-HT levels are primarily regulated by the serotonin transporter (SERT), which mediates the reuptake of extracellular 5-HT into the presynaptic nerve terminal (Murphy et al., 1998). In humans, the promoter region of the SERT gene bears a common 44-base pair deletion polymorphism (5-HTTLPR) (Lesch & Gutknecht, 2005), resulting in a 40% decrease of 5-HT reuptake in blood platelets (Greenberg et al., 1999). Despite the trait-like involvement of central 5-HT in impulsivity and aggression, human polymorphism linkage studies are inconclusive in this respect (Retz et al., 2004; Clark et al., 2005; Haberstick et al., 2006; Roiser et al., 2006). This may partially relate to disease-related factors confounding the linkage. To control for this limitation, genetic animal models may provide an alternative to study the relationship between constitutive changes in 5-HT levels and inhibitory control.

In support of such a relationship, mice lacking the SERT display reduced aggressive behaviour (Holmes et al., 2002). These mice have not been tested for impulsive behaviour or behavioural flexibility, and therefore little is known about the consequences of constitutive disturbances in SERT function and central 5-HT levels for these behaviours. Although, currently, an increasing number of studies have employed mice in operant tasks measuring executive functions (e.g. Isles et al., 2003; Pattij et al., 2003; Patel et al., 2006), the neural correlates underlying executive functioning are more extensively studied and understood in rats (Dalley et al., 2004).
Recently, we generated a SERT knockout rat (Smits et al., 2006) that completely lacks functional SERT, resulting in nine-fold increased extracellular 5-HT levels (Homberg et al., 2007). The purpose of the present experiments was to evaluate the effects of partial and complete absence of the SERT in the rat on behaviours involving inhibitory control, behavioural flexibility and aggression.

Materials and methods

Subjects

The SERT knockout rat (Slc6a4^Hhubr) was generated by target-selected ENU-induced mutagenesis [for detailed description, see Smits et al. (2006)] on a Wistar (Wistar/Crl) background. Experimental animals were generated from incrosses of heterozygous SERT^+/− rats that have been outcrossed for at least six generations. We compared as much as possible wild-type and mutant littermates. At the age of 3 weeks, ear cuts were taken under isoflurane anaesthesia and used for genotyping. Animals were socially housed at controlled room temperature (21 ± 2 °C) and relative humidity of 60 ± 15%. Water was available ad libitum throughout all experiments. Food availability and dark/ light conditions were adapted for each experiment as specified.

All experiments were conducted with the approval of the animal ethical committees of the Royal Dutch Academy of Sciences, Vrije Universiteit Amsterdam, and the University of Groningen, The Netherlands, according to the Dutch Law on animal experiments.

Resident–intruder test

Animals were housed in groups of three to four from weaning until the start of the experiments under a fixed 12 h light/dark period (lights off at 1 p.m.) and received ad libitum food. Aggression tests were performed in the dark phase between 2 p.m. and 5 p.m. [for detailed description of resident–intruder test, see De Boer et al. (1999)]. In short, at age 130–140 days the male rats were individually housed in large observation cages (80 × 55 × 50 cm), each with a sterilized female. After 1 week, the baseline level of offensive behaviour was tested on three consecutive days during a 10 min confrontation with an unfamiliar male conspecific (intruder) in the home territory of the experimental (resident) rat. Approximately 60 min prior to the start of the confrontation, the female partner of the experimental resident rat was removed from the observation cage. The naive intruder-rats were socially housed in groups of seven animals. During these three tests, only the latency time to the first full attack was recorded. On the fourth day, animals were intruder-challenged again, and from the videotaped test session (10 min after the first attack) the full range of the following behavioural elements were blindly and manually scored on a data acquisition system: (i) offensive behaviour (lateral threat, clinching, keep down, chasing, upright posture); (ii) attack latency; (iii) social exploration (moving towards, nosing, investigating opponent, ano-genital sniffing, crawl over, attempted mount, social groom); (iv) nonsocial exploration (ambulation, rearing, sniffing, scanning, digging); (v) inactivity (sitting, lying, immobile, freezing); and (vi) grooming (washing, shaking, scratching). The different behavioural elements were expressed as a percentage of the total duration of the confrontation.

Five-choice serial reaction time task

Five-choice serial reaction time task (5-CSRTT) experiments were conducted in rat operant chambers with stainless steel grid floors (model MED-NPW-5L; Medical Associates Inc., St Albans, VT, USA) housed in sound-insulating and ventilated cubicles. An array of five circular holes 2.54 cm in diameter, 2.2 cm deep and 2.25 cm above floor level was set in the curved wall of each box. Each hole was equipped with an infrared detector located across each nose poke unit 1.0 cm from the front, and a yellow LED stimulus light (6.4 mm in diameter). Rodent food pellets (45 mg, Formula P; Research Diets Inc., New Brunswick, NJ, USA) could be delivered at the opposite wall via a dispenser. In addition, the chamber could be illuminated by a white house light. The male rats were housed under a reversed dark/light cycle (lights on at 7 p.m.), and maintained at approximately 90% of their free-feeding weight, starting 1 week prior to the beginning of the experiment. Five sessions were scheduled per week from Monday until Friday, one session per day.

A detailed description of training in the 5-CSRTT has been reported previously (Van Gaalen et al., 2006). In brief, rats were trained to detect and respond to a brief visual stimulus in any one of five holes in order to obtain a food reward. Each session terminated after 100 trials or 30 min, whichever occurred first. Initially the duration of this stimulus was 32 s, and it was gradually decreased to 1 s over sessions until animals reached stable baseline performance (accuracy >80% correct choice and <20% errors of omission). The stimulus duration of 1 s was chosen because SERT^−/− rats were created on a Wistar background, an albino strain with poorer visual acuity. Previous findings in our laboratory (e.g. Van Gaalen et al., 2006; Pattij et al., 2007) have shown that with the use of this stimulus duration, similar levels of baseline performance can be attained in comparison with shorter stimulus durations of 0.5 s in nonalbino rat strains such as Lister Hooded rats (e.g. Dalley et al., 2002; Chudasama et al., 2003; Winstanley et al., 2003). Incorrect, premature responses and errors of omission did not lead to the delivery of a food reward and resulted in a 5 s time-out period during which the house light was extinguished, whereas perseverative responses, i.e. repeated responding during the presentation of the stimulus, were measured but did not have any programmed consequences. The following behavioural measures were recorded to assess task performance: (i) accuracy, i.e. percentage correct responses ([number correct trials/(correct + incorrect trials)] × 100); (ii) latency of correct responses, i.e. the mean time between stimulus onset and a correct response; (iii) premature responses, i.e. number of responses into any of the holes during the intertrial interval period and before stimulus onset; (iv) perseverative responses, i.e. the number of responses after correct choice during stimulus presentation or limited hold period; (v) the number of omissions, i.e. number of omitted trials during a session; and (vi) feeder latency, i.e. the latency between correct choice and collection of the food pellet.

Serial reversal learning

Serial reversal learning experiments were conducted in operant testing chambers (Medical Associates Inc.) that were fitted with a red house light and two small 2.5 cm square nose poke holes, separated from each other by 15 cm. A yellow stimulus light was located inside each nose poke hole. In between the nose poke units, rodent food pellets could be delivered by a dispenser (45 mg; Research Diets Inc.). On-line control of all operant chambers and data collection were performed using MED-PC version IV (Medical Associates Inc.). Rats were housed under a reversed dark/light cycle (lights on at 7 p.m.) and maintained at approximately 90% of their free-feeding weight, starting 1 week prior to the beginning of the experiment. Five sessions
were scheduled per week from Monday until Friday, one session per day.

The reversal learning procedures were modified from De Bruin et al. (2000). During the first phase, animals learned to discriminate between an inactive and active nose poke hole and had to poke for food under a fixed-ratio 3 schedule of reinforcement. Rats received 50 trials in total, and at the beginning of each trial, the yellow stimulus lights inside both the right and left nose poke holes were illuminated. Nose poking into the relevant hole resulted in immediate delivery of a food reward and extinguished the stimulus lights inside both holes, whereas nose poking into the irrelevant hole extinguished the stimulus lights and did not result in food reward. Alternatively, when the animal did not poke during a trial, the stimulus lights extinguished after 30 s without delivery of a food pellet. A new trial started with an intertrial interval ranging from 5 to 25 s (mean 15 s) after a response into the food cup or a 30 s time-out period, whichever occurred first. When the animals fully mastered discrimination learning (>90% correct choices), the task demands were reversed. To this end, in subsequent sessions the other nose poke hole was reinforced until criterion performance was reached again. In total, rats received four reversals, and the percentage correct responses ([number correct trials/(correct + incorrect trials)] × 100) was calculated for acquisition of spatial discrimination and the subsequent four reversals.

**Monoamine levels in brain tissue and CSF**

Male rats were housed under normal light/dark conditions (lights on at 7 a.m.) and received ad libitum food. Thus, neurochemical analyses were conducted during the light phase, in contrast to the behavioural experiments. With regard to the circadian rhythmicity in monoamine levels, including 5-HT, which are generally elevated during waking (dark phase) (Portas et al., 2000), the current measurements of 5-HT in tissue and CSF may be an underestimation of these levels during waking in all genotypes. To obtain medial prefrontal cortex (mPFC), orbitofrontal cortex (OF), lateral hypothalamus (LH) and raphe nuclei (RN) tissue, rats were decapitated and their brains were rapidly removed by dissection on an ice-plate. From 2.20 to 3.20 mm rostral to bregma (Paxinos & Watson, 1998), the prelimbic and infralimbic cortices corresponding to the mPFC, as well as the OFC were dissected. From −0.60 to −1.60 mm rostral to bregma, the lateral hypothalamus was dissected, and from −7.20 to −8.28 mm, the median and dorsal RN. Tissue from the left and right hemispheres were combined and immediately frozen on dry-ice and subsequently stored at −80 °C until use. Brain tissue was sonicated in 1 mL of 0.5 M perchloric acid, and centrifuged at 14,000 g for 10 min at 4 °C; 100 µL of supernatant was used for high-performance liquid chromatography (HPLC) analysis.

For CSF measurements, isoflurane-anesthetized rats were fixed in a stereotaxic such that the body hung downwards at an angle of 90 °C with respect to the head. A 5 mm needle was inserted into the cisterna magna via the back head hole, and CSF (~50 µL) was collected and stored at −20 °C until HPLC analysis.

CSF levels: 20 µL samples were injected via an autoinjector (Agilent 1100) onto a 150 × 4.6 mm LC18DB Supelcosil 3 µm diameter column (kept at 20 °C) and eluted with 50 mM isocratic ammonium phosphate buffer (pH 2.5) containing 0.1 mM EDTA, 1.5 mM sodium octylsulphate, 100 mM sodium perchlorate and 1.5% n-propanol. The HPLC outlet was connected to an electrochemical detection chamber (Decade model 400; Antec, Leiden, The Netherlands) using a glassy carbon fibre electrode set to 410 mV as compared to the reference Ag/AgCl Vt-3 electrode. Oxidation potentials were recorded and 5-HT levels were quantified using L-methyserotonin, which was added to each sample as external reference.

Brain tissue levels: 100 µL samples were injected into an HPLC column (Gemini C18 110A, 150 × 4.60 mm, 5 µm; Bester) connected to a detector (analytical cell: ESA model 5011, 0.34 V). The mobile phase consisted of 62.7 mM Na2HPO4, 40 mM citric acid, 0.27 mM EDTA, 4.94 mM human serum albumin (HSA) and 10% MeOH (pH 4.1). Known amounts of the monoamines and metabolites were run in parallel as external standards.

**Statistical analyses**

Data were subjected to single or repeated measures analysis of variance (ANOVA) with genotype as between-subjects variable. The homogeneity of variance across groups was determined using Mauchly’s tests for equal variances and in case of violation of homogeneity, corrected, and therefore more conservative Huynh–Feldt probability values were used for subsequent analyses. Statistically significant main genotype effects were further analysed using the Student–Newman–Keuls test, whereas paired-sample t-tests were used to assess within-genotype effects. The level of probability for statistically significant effects was set at \( P < 0.05 \).

**Results**

**Reduced aggressive behaviour in SERT−/− rats**

In the resident–intruder test, rats display territorial aggressive behaviour towards an unfamiliar intruder to defend their home cage territory. Over all encounters, SERT−/− rats were more reluctant to initiate attack than SERT+/+ and SERT+/− rats (Fig. 1A; \( F_{2,30} = 7.07, P = 0.039 \)). Furthermore, during the fourth encounter, SERT−/− rats spent less time on offensive behaviour (Fig. 1B; \( F_{2,29} = 0.47, P = 0.012 \)), whereas nonaggressive social behaviours and nonsocial behaviours were similar (Table 1).

**Improved inhibitory control in SERT−/− rats**

All genotypes acquired stable baseline performance in the 5-CSRTT after approximately 25 training sessions. Therefore, data are depicted from session 25 onwards for 10 consecutive baseline training sessions. With regard to measures of attentional function, there were no differences in the levels of accurate responding between genotypes over sessions (Fig. 2A; session, \( F_{9,297} = 1.25, P = 0.25 \); genotype × session, \( F_{18,297} = 0.91, P = 0.57 \); and genotype, \( F_{2,33} = 0.31, P = 0.74 \)). Nonetheless, correct response latencies were...
longer in SERT–/– rats (Fig. 2B; session, $F_{9,297} = 0.81, P = 0.60$; genotype $\times$ session, $F_{18,297} = 1.33, P = 0.17$; and genotype, $F_{2,33} = 12.18, P < 0.001$), whereas SERT+/+ animals behaved like SERT+/– animals. The number of premature responses was lower in SERT–/– rats in comparison with SERT+/+ and SERT+/– rats, although there was some between-session variation in all genotypes.

Table 1. Social and nonsocial behaviours in resident–intruder test

<table>
<thead>
<tr>
<th></th>
<th>SERT+/+ rats</th>
<th>SERT+/– rats</th>
<th>SERT–/– rats</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social exploration (%)</td>
<td>13 ± 2.8</td>
<td>11 ± 2.5</td>
<td>20 ± 3.9</td>
<td>$F_{2,30} = 2.07, P = 0.144$</td>
</tr>
<tr>
<td>Social interaction (%)</td>
<td>21 ± 2.9</td>
<td>24 ± 3.6</td>
<td>21 ± 3.9</td>
<td>$F_{2,30} = 0.33, P = 0.722$</td>
</tr>
<tr>
<td>Non-social (%)</td>
<td>51 ± 5.0</td>
<td>56 ± 4.1</td>
<td>54.5 ± 4.7</td>
<td>$F_{2,30} = 0.30, P = 0.745$</td>
</tr>
<tr>
<td>Immobility (%)</td>
<td>16 ± 4.5</td>
<td>13 ± 3.8</td>
<td>11.6 ± 4.2</td>
<td>$F_{2,30} = 0.32, P = 0.725$</td>
</tr>
<tr>
<td>Grooming (%)</td>
<td>12 ± 5.1</td>
<td>6.3 ± 2.4</td>
<td>13 ± 3.1</td>
<td>$F_{2,30} = 0.89, P = 0.422$</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. SERT, serotonin transporter.

Fig. 2. Premature responding in the 5-CSRTT is reduced in SERT–/– rats. Data depict mean (± SEM) percentage of accurate responding (A), latency to make a correct choice (B), number of premature responses (C) and number of perseverative responses after correct choice (D) during 10 consecutive baseline sessions.
contributing to the observed main session effect (Fig. 2C; session, F(2,29) = 2.56, \( P = 0.014 \); genotype \( \times \) session, F(18,297) = 0.61, \( P = 0.86 \); and genotype, F(2,33) = 4.18, \( P = 0.024 \)). In contrast, the number of perseverative responses did not differ between genotypes (Fig. 2D; session, F(2,29) = 1.57, \( P = 0.17 \); genotype \( \times \) session, F(18,297) = 1.40, \( P = 0.18 \); and genotype, F(2,33) = 0.25, \( P = 0.78 \)). There were no genotype differences in the number of omissions, although omissions significantly declined over sessions from approximately 25–20 omissions (session, F(2,29) = 5.16, \( P < 0.001 \); genotype \( \times \) session, F(18,297) = 1.33, \( P = 0.19 \); and genotype, F(2,33) = 0.60, \( P = 0.56 \). Lastly, feeder latencies were not different between genotypes (session, F(2,29) = 1.53, \( P = 0.18 \); genotype \( \times \) session, F(18,297) = 1.22, \( P = 0.28 \); and genotype, F(2,33) = 0.69, \( P = 0.51 \)).

Cognitive flexibility unchanged in SERT\(^{-/-}\) rats

In the serial reversal learning paradigm, rats from all three genotypes readily learned to discriminate between relevant and irrelevant stimulus to criterion performance within three sessions (accurate choice >90%) with no differences in discrimination learning between genotypes (session, F(3,32) = 1.75, \( P = 0.001 \); genotype \( \times \) session, F(9,297) = 1.16, \( P = 0.34 \); and genotype, F(2,21) = 2.03, \( P = 0.16 \)). Changing the task demands by stimulus reversal revealed no significant differences between genotypes in switching to the previously irrelevant stimulus (Fig. 3; reversal 1, F(2,29) = 0.17, \( P = 0.84 \)). Also in the subsequent three reversal sessions, statistical analyses revealed no significant differences between genotypes in their ability to switch from relevant to previously irrelevant stimulus (reversal 2, F(2,29) = 1.55, \( P = 0.23 \); reversal 3, F(2,29) = 0.90, \( P = 0.42 \); and reversal 4, F(2,29) = 1.76, \( P = 0.19 \)), although the ability to switch strategy improved over subsequent reversal sessions for all genotypes (session, F(3,78) = 55.97, \( P < 0.001 \); genotype \( \times \) session, F(6,78) = 1.54, \( P = 0.19 \); and genotype, F(2,26) = 0.084, \( P = 0.92 \)).

Reduced levels of 5-HT, but not other monoamines, in mPFC, OFC, LH, RN and CSF of SERT\(^{-/-}\) rats

Neurochemical analyses indicated that 5-HT levels were significantly reduced in the mPFC, OFC, LH and RN of SERT\(^{-/-}\) rats, along with 5-HIAA, whereas 5-HIAA/5-HT ratios were unchanged (Table 2). On the other hand, dopamine (DA), noradrenaline, dihydroxyphenylacetic acid and homovanillic acid levels were not different between genotypes. In the CSF of SERT\(^{-/-}\) rats, 5-HT levels were reduced, whereas DA and dihydroxyphenylacetic acid levels were unchanged. Furthermore, the 5-HIAA/5-HT ratio in CSF was significantly enhanced in SERT\(^{-/-}\) rats.

Discussion

The present data indicate that genetic ablation of the SERT in the rat results in reduced aggressive behaviour in a resident–intruder paradigm, as well as improved inhibitory control in the 5-CSRTT. In contrast, cognitive flexibility as measured in a visuospatial discrimination and reversal learning paradigm was not altered in SERT\(^{-/-}\) rats. In line with our previous observations (Homberg et al., 2007), neurochemical analyses revealed that mPFC, OFC, LH and RN 5-HT levels in tissue were reduced in SERT\(^{-/-}\) rats, whereas DA and noradrenaline levels were unchanged. Together, these findings strongly suggest a selective involvement of the serotonergic system in the observed changes in aggression and inhibitory control.

In both SERT\(^{-/-}\) mice (Mathews et al., 2004) and rats (Homberg et al., 2007), extracellular 5-HT levels were found to be increased. The reduced 5-HT tissue levels in combination with the increased extracellular 5-HT levels may seem contradictory, but both are a direct consequence of the absence of SERT in the SERT\(^{-/-}\) rats. This absence results in reduced 5-HT reuptake and consequently in reductions in tissue levels of 5-HT and 5-HIAA along with increments in extracellular 5-HT levels. However, as different mechanisms may contribute to disturbed 5-HT levels in humans, one cannot generalize the observed relationship between intracellular and extracellular 5-HT levels – other manipulations such as 5-HT lesions or treatment with 5-HT-releasing agents do not necessarily result in opposing extracellular and tissue monoamine levels (e.g. Clarke et al., 2004). Furthermore, most studies on aggression and impulsivity include only measurements of either extracellular or intracellular 5-HT levels, complicating a direct comparison with our results.

An interesting finding in the present study was the observation that 5-HT levels in CSF were significantly reduced and the 5-HIAA/5-HT ratio in CSF was increased in SERT\(^{-/-}\) knockout rats. This suggests that 5-HT turnover is increased in SERT\(^{-/-}\) rats, which confirms that the serotonergic tone in this mutant rat model is increased. In support of previous data indicating elevated extracellular 5-HT in SERT\(^{-/-}\) rats and thus tonically elevated 5-HT neurotransmission (Homberg et al., 2007), the CSF measurements and extracellular 5-HT levels seem to correspond in terms of central serotonergic activity. This notion is very valuable in the interpretation of CSF 5-HIAA measurements in humans and nonhuman primates in relation to central serotonergic activity. (Linnola et al., 1983; Fairbanks et al., 2001).

Consistent with previous findings in SERT\(^{-/-}\) mice (Holmes et al., 2002), the present findings show that attack latencies and offensive behaviour, but not other aggression-unrelated social behaviours, were reduced in SERT\(^{-/-}\) rats as compared to wild-type rats. Together, these comparable findings across species indicate an important modulatory role of the serotonergic system in the inhibition of aggression. Furthermore, there was a selective decrease in 5-HT levels in the lateral hypothalamus, which is considered a major constituent of the ‘hypothalamic aggression area’ (Roeling et al., 1994). These observations
support the notion that 5-HT neurotransmission in this brain region is crucially involved in controlling aggressive behaviour (Kantak et al., 1984). However, we cannot exclude the contribution of other brain regions in this regard. Moreover, whether extracellular 5-HT levels are elevated in the lateral hypothalamus remains to be determined.

Until now, very little has been known about the role of SERT in impulsivity, and data from human SERT polymorphism linkage studies are inconclusive in this respect. Whereas in some of these studies an association between the short allelic version of 5-HTTLPR and aggressive and violent behaviour or aspects of impulsivity has been reported (Retz et al., 2004; Haberstick et al., 2006; Roiser et al., 2006), other studies have not found such an association (Clark et al., 2004; Passetti et al., 2006), whereas administration of the 5-HT-releasing agent d-fenfluramine has been shown to decrease premature responding in the 5-CSRTT (Carli & Samanin, 1992). In addition to reduced premature responding in the 5-CSRTT, correct response latencies were lengthened in SERT−/− rats as compared to both other genotypes. This may suggest that reduced locomotor activity partly explains the observed differences in inhibitory control. However, other parameters such as feeder latencies and omission were not changed in SERT−/− rats. Likewise, home cage and open field locomotor activity were not different between SERT−/− and wild-type rats (unpublished observations), suggesting that the improvements in inhibitory control in SERT−/− rats are not solely attributable to changes in general locomotor activity.

Lesion studies have stressed the importance of the mPFC in inhibitory control in the 5-CSRTT (Muir et al., 1996; Chudasama et al., 2003). In this regard, the observation that tissue levels of 5-HT, and not other monoamines such as DA, were reduced in the mPFC of SERT−/− rats is interesting. Although we did not measure extracellular 5-HT levels in these studies infusions of 5-HT2 receptor antagonists into the mPFC in inhibitory control as measured in the 5-CSRTT (Passetti et al., 2003; Winstanley et al., 2003). However, it should be noted that in these studies infusions of 5-HT2 receptor antagonists into the mPFC
were found to reduce premature responding. Thus, if tonic 5-HT neurotransmission in SERT−/− rats is elevated in the mPFC in addition to the hippocampus (Homberg et al., 2007), our findings are not entirely consistent with these results. Clearly, future experiments that focus on compensatory adaptations in postsynaptic 5-HT receptor subtypes, including 5-HT2 receptor subtypes, are needed to compare the present findings with existing literature.

Integrity of the OFC is critical for flexible responding in reversal learning in rats (Boulougouris et al., 2007), and reduced extracellular and intracellular 5-HT levels in the marmoset monkey (Clarke et al., 2004, 2005) as well as reduced OFC 5-HT tissue levels in rats (Masaki et al., 2006) have been demonstrated to correlate with impaired reversal learning. In addition, acute tryptophan depletion in humans (Rogers et al., 1999) has been shown to robustly impair reversal learning. Accordingly, in keeping with these observations, we expected that the putative increased extracellular OFC 5-HT levels in SERT−/− rats would correlate with improvements in behavioural flexibility. Nonetheless, in the current study we did not observe genotype differences in behavioural flexibility. Although the reversal learning paradigm employed in the present study is a simplified version of the tests used in monkeys (Clarke et al., 2004, 2005) and humans (Rogers et al., 1999) and did not assess probabilistic reversal learning, the percentage correct responses during the first reversal session was low in all animals. These observations may indicate that, particularly in the first reversal session, there was sufficient room for improvement in behavioural performance. Our findings thus suggest that there is a possible ceiling effect for the modulatory role of 5-HT in behavioural flexibility, whereby increasing the serotonergic tone would not further improve behavioural performance in the reversal learning paradigm. This remains speculative, however, because we did not measure extracellular 5-HT levels in the OFC.

In conclusion, the present findings indicate that genetic ablation of the SERT in rats is associated with reduced aggressive behaviour and improved inhibitory control along with selective adaptations in the 5-HT system in various brain regions and CSF. In humans, the therapeutic efficacy of selective serotonin reuptake inhibitors in aggressive encounters and other impulse control disorders (e.g. Coccaro & Kavoussi, 1997; Brady et al., 1998) indicates that inhibition of SERT function alleviates symptoms that are associated with these disorders. In this regard, the current behavioural observations in SERT−/− rats nicely fit with the clinical findings. The SERT knockout rat may therefore be a useful tool to further study the role of 5-HT homeostasis in impulse control disorders, as constitutive changes in central 5-HT function are thought to contribute to personality factors such as coping style and impulsivity (Serretti et al., 2006; Highley & Linnoila, 1997).

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Abbreviations

CSF, cerebrospinal fluid; 5-CSRTT, five-choice serial reaction time task; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine; LH, lateral hypothalamus; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; RN, raphe nuclei; SERT, serotonin transporter.

References


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