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Serotonin transporter dosage modulates long-term decision-making in rat and human

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ABSTRACT

Decision-making plays an important role in everyday life and is often disturbed in psychiatric conditions affected by the common human serotonin transporter promoter length polymorphism (5-HTTLPR). This raises the hypothesis that decision-making is modulated by the serotonergic system, but currently it is unclear how the 5-HTTLPR affects central serotonergic functioning. We tested healthy human volunteers genotyped for the 5-HTTLPR in the Iowa Gambling Task (IGT), which is one of the most frequently used neuropsychological tasks to assess decision-making. Furthermore, we tested female homozygous ($SERT^{-/-}$) and heterozygous ($SERT^{+/-}$) serotonin transporter knockout rats in a rodent version of the IGT. Women homozygous for the short (s) allele of the 5-HTTLPR were found to choose more disadvantageously than women homozygous for the long allele of the 5-HTTLPR as the IGT progressed. In the rat, $SERT^{-/-}$ and $SERT^{+/-}$ were associated with advantageous decision-making compared to $SERT^{+/+}$ as the IGT progressed. Combining the human and rat observations, we show that SERT dosage affects the maintenance of a once established choice option, irrespective of the choice (advantageous or disadvantageous) that has been made. We postulate that the SERT-mediated effects relate to deficits in the processing of choice outcome to guide subsequent choices in this gamble-based test, and that $SERT^{-/-}$ and $SERT^{+/-}$ rodent models in combination with studies in humans can be used to provide insight in the modulatory effects of 5-HTTLPR.

Decision-making is essential to everyday life and serious problems can develop when disturbed (Bechara et al., 1994). Understanding its neurobiological mechanisms may be a key to the treatment of several psychiatric disorders. The Iowa Gambling Task (IGT) is the most frequently used task to assess decision-making performance under uncertainty (Bechara et al., 1994, 1999). The IGT requires individuals to choose between four different decks of cards, which are either advantageous in the long run because cards not only contain moderate monetary gains but also moderate or low loses, or disadvantageous in the long run because even though gains are high, the losses are even higher. Usually, humans change their choice behavior such that in the second half of the task they make substantially more choices for the option with the best long-term outcome (Bechara et al., 1994, 1999; Brand et al., 2007b). The amygdala and several frontal cortical regions, including the ventromedial prefrontal cortex (vmPFC) and anterior cingulate (ACC), play a central role in this gamble-based decision-making (Bechara et al., 1999; Tucker et al., 2004; Brand et al., 2007a; Fellows and Farah, 2005; Hsu et al., 2005; Manes et al., 2002). The well-known serotonin transporter-linked polymorphic region (5-HTTLPR) is associated with variation in the functional coupling between these regions (Heinz et al., 2005; Pezawas et al., 2005). This, and other data (Bechara et al., 2001), raise the hypothesis that serotonin (5-hydroxytryptamine, 5-HT) modulates decision-making, possibly by maintaining a once established choice option (Van den Bos et al., 2006a).

The serotonin transporter (SERT) reuptakes extra-neuronal 5-HT. Lymphoblast studies showed that the short (s) allelic variant of the 5-HTTLPR is associated with reduced SERT expression and function as compared to the long (l) allele (Lesch et al., 1996). However, in the brain, SERT mRNA levels (Jim et al., 2006) and SERT binding (e.g. Parsey et al., 2006) have not been found to be related to 5-HTTLPR genotype. Although understanding how the 5-HTTLPR affects the functioning of the serotonergic system is crucial for interpreting individual differences in decision-making and psychiatric disease susceptibility, such studies are severely hampered

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by the limited experimental interventions that are possible in humans. For serotonin transporter knockout ($SERT^{-/-}$) rodent models it is, to a large extent, known how the serotonergic system is functioning. We argue here that this information can be used to understand the mechanisms underlying 5-HTTLPR phenotypes, if comparable tests in rodents and humans are used. The availability of a rodent version of the IGT makes this possible (Van den Bos et al., 2006b). In this study we used the novel SERT homozygous ($SERT^{-/-}$) and heterozygous ($SERT^{+/-}$) rat knockout model (Smits et al., 2006), for which we know that extra-neuronal 5-HT levels are gene-dose dependently increased (Homberg et al., 2007a; J.H., E.C., unpublished observations). The aim of this study was two-fold: (1) to study the effect of genotype-dependent SERT dosage on decision-making as measured in the IGT in humans and rats, and (2) to study whether this effect is comparable in humans and rats.

1. Material and methods

1.1. Healthy female volunteers

Female subjects were recruited at the University campus. For their participation they were awarded 10 Euro as well as included in a lottery for winning an iPod, to be held when all subjects had been tested. They were all healthy, with no history of or current psychiatric disorder or past or present use of drugs as determined by a questionnaire. As we were also interested in any potential eating disorders in our study population, we recruited subjects with a body mass index (BMI) between 19 and 24, i.e. within a normal range of bodyweight (see further below). Subjects were tested during the follicular phase (Van den Bos et al., 2007).

1.2. SERT knockout rats

The SERT knockout rat was generated by ENU (*N*-ethyl-*N*-nitrosurea)-induced mutagenesis (Smits et al., 2006). Experimental animals were obtained from crosses between heterozygous SERT knockout rats that were outcrossed for five generations. In all experiments female $SERT^{+/+}$, $SERT^{+/-}$ and $SERT^{-/-}$ littermates were compared. At the age of 3 weeks ear cuts were taken for genotyping. Animals were socially housed in enriched type IV macrolon cages under a reversed 12 h light/dark cycle (lights on at 7 pm) at controlled room temperature (21 ± 2 °C) and relative humidity of 60 ± 15%. The animals were tested in batches between the age of 10 and 14 weeks. The experiments were conducted with approval of the animal ethical committees of the Utrecht Medical Center, and the Royal Dutch Academy of Science, the Netherlands.

1.3. Iowa Gambling Task in healthy females

Females were subjected to a series of tests and questionnaires of which the IGT was the first. The other tests and questionnaires were related to eating disorders, and the outcome related to the polymorphism will be reported elsewhere. The original human IGT (Bechara et al., 1994) was automated using Microsoft Excel with VBA macros and performed as described previously (Van den Bos et al., 2006a). As in previous studies it turned out that subjects improve their performance after 40 trials (Bechara et al., 1994; Van den Bos et al., 2006a), we analyzed trials 1–40 and 41–100 separately (Brand et al., 2007b). We subtracted the number of disadvantageous (A and B) choices from the number of advantageous (C and D) choices for trials 1–40 and 41–100, and divided the difference score by 2 (trials 1–40) or 3 (trials 41–100). At the start of the experiment, buccal cells for 5-HTTLPR genotyping were collected *in duplo* using cotton swabs (BIOzymTC, Madison, Wisconsin). All subjects gave informed consent for obtaining DNA material and 5-HTTLPR genotyping.

1.4. 5-HTTLPR genotyping

Genomic DNA was extracted from the buccal cells using a BuccalAmp™ DNA extraction kit (BIOzymTC, Madison, Wisconsin) according to manufacturer's recommendations. The 5-HTTLPR fragments were amplified using a 6FAM fluorescently labeled forward primer (5′-CAG CAA CTC CC TGT ACC C-3′) and an unlabeled reverse primer (5′-GGA GAT CCT GGG AGA GGT G-3′) in a PCR cycling program consisting of 20 cycles of 92 °C for 20 s, 57 °C for 20 s and 72 °C for 30 s; 72 °C for 180 s (GeneAmp9700, Applied Biosystems, Foster City CA, USA). The PCR reactions contained 5 μl DNA, 0.2 μM forward primer, 0.2 μM reverse primer, 400 μM of each dNTP, 25 mM tricine, 7% glycerol (w/v), 1.6% DMSO (w/v), 2 mM $MgCl_2$, 85 mM ammonium acetate (pH 8.7), and 0.2 U Taq polymerase in a total volume of 10 μl. The PCR products were purified using acetate/ethanol precipitation and dissolved in 30 μl MilliQ-grade water. One microliter purified PCR product was mixed with 0.05 μl GeneScan 500 LIZ size standard (Applied Biosystems) in 10 μl MilliQ-grade water, denatured for 5 min at 95 °C, and subsequently run on an ABI3730XL capillary sequencer (Applied Biosystems). Product lengths were analyzed using Genemapper software (Applied Biosystems).

1.5. Iowa Gambling Task in female SERT knockout rats

The Iowa Gambling Task was performed as described previously (Van den Bos et al., 2006b) with minor modifications. In short, food restricted rats (95 ± 5% free feeding body weight) were tested between 10.00 am and 16.00 pm. A trial started by lifting the slide door of the start box and the rat could freely explore the apparatus and eat the pellets for 2 min at maximum. The inter-trial interval was 15 s. The rats received 10–20 trials/day for a total of 120 trials. Rewards were represented by sucrose pellets, punishments were represented by quinine-treated sucrose pellets that were unpalatable but not uneatable. The 'bad' arm was represented by occasionally big rewards (three sucrose pellets) among series of quinine-treated pellets (9/10 positions). The 'good' arm was represented by regularly small rewards (one sucrose pellet) among quinine-treated sucrose pellets (2/10 positions). Two empty arms were included to measure non-reward related effects. The rewarded and empty arms, as well as 'good' and 'bad' arms were counterbalanced across subjects. The total number of visits to 'good' and 'bad' arms was calculated for the first half (trials 1–60) and the second half of the test (trials 61–120) and the number of visits to the 'bad' arm were subtracted from the number of visits to the 'good' arm to obtain the IGT score. To compare the rat data to the human data, the difference score was divided by 3. The choices for the empty arms were analyzed as proportion of empty arm visits per block of 10 trials (Van den Bos et al., 2006b).

1.6. Statistics

Data were checked for normality and homogeneity. Both in humans and in rats, overall IGT analysis was performed using two-way analysis of variance (ANOVA) for repeated measures (trial block). Significant main effects were followed by a Student–Newman–Keuls (SNK) test and a separate one-way ANOVA per trial block. Significant interactions were followed by a paired Student's *t*-test. IGT score was compared to the 0 score (advantageous–disadvantageous choices per 20 trials = 0) using a one-sample Student's *t*-test. Differences were considered significant when $P \leq 0.05$; NS = non-significant. All statistics are two-tailed.

2. Results

2.1. IGT in humans

Of the 89 healthy female human subjects one individual was discarded since she had a BMI > 24. Subjects were aged between 19 and 31 (average age ± S.E.M.: 22.2 ± 0.3). The l/l ($n = 17$), l/s ($n = 49$) and s/s ($n = 22$) genotype distribution were in Hardy–Weinberg equilibrium [$\chi^2 = 1.21$, df = 1, NS] and alleles were found to be normally distributed over age [$F(2, 85) = 0.771$, NS].

As can be seen in Fig. 1, independent of genotype all subjects chose more often advantageously from trials 1–40 to trials 41–100 as demonstrated by a strong trial block effect [$F(1, 85) = 37.41$, $P < 0.0001$] with no genotype × trial block interaction [$F(2, 85) = 0.67$, NS]. However, overall s/s subjects chose more often

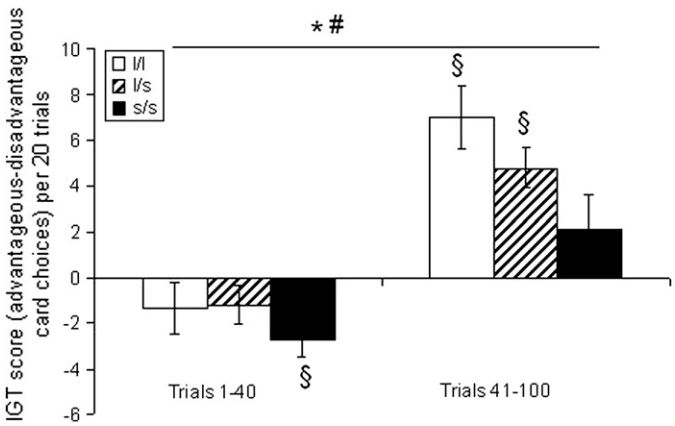

Fig. 1. Female healthy subjects with the l/s and l/l genotype choose the long-term advantageous decks more often than s/s subjects as the human IGT progresses. Shown is the mean number (±S.E.M.) of advantageous choices minus disadvantageous choices over the first phase (trials 1–40) and the second phase (trials 41–100) in l/l ($n = 17$), l/s ($n = 49$) and s/s ($n = 22$) female volunteers per 20 trials. *s/s Different from l/l and l/s, $P < 0.05$; §different from a zero IGT score (advantageous–disadvantageous = 0), $P < 0.05$; and #significant improvement from trials 1–40 to trials 41–100, $P < 0.05$.

disadvantageously than l/s and l/l subjects [post hoc SNK ($P < 0.05$) following a significant genotype effect: $F(2, 85) = 4.33$, $P < 0.02$]. Closer inspection of the data in Fig. 1 suggests that genotype effects are stronger in trial blocks 41–100 than trial blocks 1–40. Indeed, separate one-way ANOVAs for trials 1–40 and 41–100 reveal a near significant genotype effect for trial blocks 41–100 [$F(2, 85) = 2.99$, $P < 0.06$] but no significant effect for trial blocks 1–40 [$F(2, 85) = 0.89$, NS]. When the IGT score was analyzed against a 0 score (advantageous–disadvantageous choices = 0) for trial blocks 1–40, l/l [$T(\mathrm{df} = 16) = 1.23$, NS] and l/s [$T(\mathrm{df} = 48) = 1.37$, NS] subjects did not show a preference for disadvantageous or advantageous decks, but s/s subjects chose significantly more often cards from the disadvantageous than advantageous decks [$T(\mathrm{df} = 21) = 3.64$, $P < 0.002$]. For trial blocks 41–100 l/l [$T(\mathrm{df} = 16) = 5.29$, $P < 0.0001$] and l/s [$T(\mathrm{df} = 48) = 5.67$, $P < 0.0001$] subjects, but not s/s subjects [$T(\mathrm{df} = 21) = 1.34$, NS], chose significantly more cards from the advantageous decks than the disadvantageous decks (Fig. 1). These results overall show that the 5-HTTLPR affects IGT performance during the second phase of the task only, and that l/l and l/s subjects strongly improved IGT performance as the test progressed, while s/s subjects did weakly so.

2.2. IGT in rats

Thirteen SERT$^{+/+}$, 14 SERT$^{+/-}$ and 10 SERT$^{-/-}$ female rats were tested in the rat version of the Iowa Gambling Task (Van den Bos et al., 2006b). Fig. 2 shows that depending on genotype rats showed an improvement from trials 1–60 to trials 61–120 as exemplified by a significant trial block effect [$F(2, 34) = 30.13$, $P < 0.0001$] and trial block × genotype interaction [$F(2, 34) = 6.02$, $P < 0.006$]. No overall genotype effect was found [$F(2, 34) = 1.75$, NS]. Both SERT$^{+/-}$ [$T(\mathrm{df} = 13) = 4.42$, $P < 0.001$] and SERT$^{-/-}$ [$T(\mathrm{df} = 9) = 4.66$, $P < 0.001$] rats clearly chose more often advantageously from trial blocks 1–60 to trial blocks 61–120, whereas SERT$^{+/+}$ rats [$T(\mathrm{df} = 12) = 2.77$, NS] did not. Comparison of the IGT score against the 0 score during trials 1–60 revealed that both SERT$^{+/+}$ [$T(\mathrm{df} = 12) = 3.80$, $P < 0.003$] and SERT$^{-/-}$ [$T(\mathrm{df} = 9) = 2.69$, $P < 0.03$] rats chose more often advantageously, while SERT$^{+/-}$ rats did not show a preference for either arm [$T(\mathrm{df} = 13) = 0.80$, NS]. During trials 61–120 SERT$^{+/+}$ [$T(\mathrm{df} = 12) = 2.21$, $P < 0.05$], SERT$^{+/-}$ [$T(\mathrm{df} = 13) = 4.59$, $P < 0.001$] and SERT$^{-/-}$ [$T(\mathrm{df} = 9) = 6.28$, $P < 0.0001$] rats all chose significantly more often advantageously (Fig. 2). These results overall show that SERT$^{+/+}$ rats showed a weaker improvement across trials than SERT$^{-/-}$ and SERT$^{+/-}$ rats.

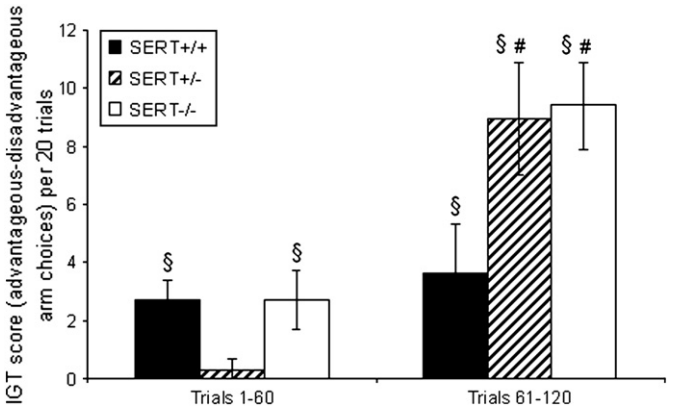

Fig. 2. Female SERT$^{-/-}$ and SERT$^{+/-}$ rats choose the long-term advantageous arms more often than SERT$^{+/+}$ rats as the rat IGT progresses. Shown is the mean number (±S.E.M.) of advantageous arm choices minus disadvantageous arm choices over the first phase (trials 1–60) and the second phase (trials 61–120) in female SERT$^{+/+}$ ($n = 13$), SERT$^{+/-}$ ($n = 14$) and SERT$^{-/-}$ ($n = 10$) rats per 20 trials. §Different from a zero IGT score (advantageous–disadvantageous = 0), $P < 0.05$; #significant improvement from trials 1–60 to trials 61–120, $P < 0.05$.

The rodent IGT also contained two empty arms to measure aspecific choice behavior. Fig. 3 shows that SERT$^{+/-}$ and SERT$^{-/-}$ rats decreased their visits to the empty arms more strongly than SERT$^{+/+}$ rats as the test progressed as exemplified by a significant trial block × genotype effect [$F(22, 374) = 1.583$, $P < 0.02$]. Like for the choices for the rewarded arms this effect was stronger for trial blocks 61–120 [trial block × genotype $F(10, 170) = 1.97$, $P < 0.04$; genotype $F(2, 34) = 2.67$, $P < 0.09$] than for trial blocks 1–60 [trial block × genotype $F(10, 170) = 1.15$, NS]. Unlike SERT$^{-/-}$ and SERT$^{+/-}$ rats, SERT$^{+/+}$ rats remained showing exploratory behavior, i.e. visited the empty arms throughout the task. These results show that while rats of all genotypes were equally exploring the available choice options during the first phase of the task, only SERT$^{-/-}$ and SERT$^{+/-}$ animals directed their choice to the baited (good and bad) arms as the task progressed.

3. Discussion

Aim 1: The IGT performance in both female human subjects and female rats was most clearly affected by SERT genotype in the second half of the IGT, supporting the hypothesis that 5-HT becomes important during the second phase of the test when a choice has been established. In support, the decrease in empty arm visits during the second phase of the test indicates that the SERT$^{-/-}$ and SERT$^{+/-}$ rats almost completely ignored the empty arms and chose for the baited arms, while SERT$^{+/+}$ rats continued to explore all possible options. Aim 2: However, while SERT$^{+/-}$ and SERT$^{-/-}$ rats made advantageous choices (more sucrose pellets on the long run), s/s subjects made disadvantageous choices (less money on the long run). The apparent dissociation between the genotype-dependent SERT dosage effect on maintaining a once established choice option on the one hand, and the nature of this choice on the other hand, may imply that the 5-HTTLPR and SERT knockout modulate a similar process in gamble-based choice behavior, independent of its outcome.

While 5-HTTLPR functional studies in lymphoblasts (Lesch et al., 1996) have shown that the s-allele is associated with reduced SERT function, which is a reason to argue that the SERT$^{-/-}$ in rodents resembles effects of the s/s genotype, it should be noted that no conclusive data exist that show that the s/s genotype is associated with decreased SERT expression in the central nervous system (CNS; e.g. Van Dyck et al., 2004; Jim et al., 2006; Parsey et al., 2006). It has even been reported that in s/s carriers compared to l/s carriers in the age category between 20 and 30 years (same age category as the subjects tested in this study) central SERT binding is increased (Van Dyck et al., 2004). Rather than SERT expression levels, it might be 5-HT-induced neurodevelopmental adaptations that are shared by SERT knockout rodents and individuals carrying the s-allele of the 5-HTTPR. During early stages of development, 5-HT also acts as trophic factor (Lauder, 1990). It has been demonstrated that SERT$^{-/-}$ in the mouse affects the structure of the (prefrontal) cortex and amygdala (Wellman et al., 2007; Altamura et al., 2007), regions that show changes in functional connectivity in relation to the 5-HTTLPR (Heinz et al., 2005; Pezawas et al., 2005).

IGT performance is dependent on the functional integrity of the amygdala and several regions of the frontal cortex, including the vmPFC and ACC (Bechara et al., 1999; Tucker et al., 2004). Healthy s-allele carriers have been reported to display an increased functional coupling between the vmPFC and the amygdala compared to l/l subjects (Heinz et al., 2005). The vmPFC and amygdala are strongly implicated in fear conditioning (LeDoux, 1996), and increased vmPFC–amygdala activation in s/s subjects may promote the acquisition of conditioned responses in the IGT. It is possible that the

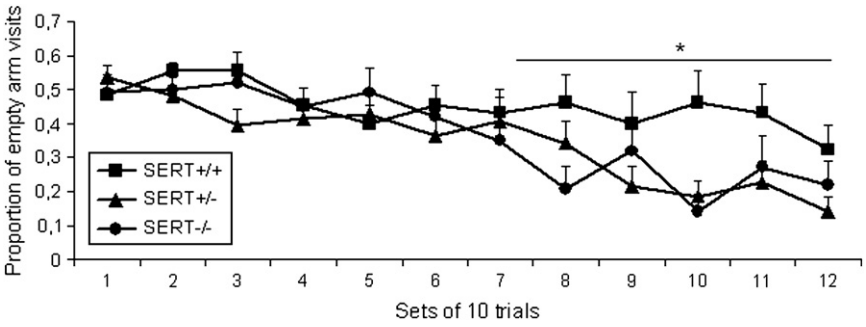

Fig. 3. Female SERT$^{-/-}$ and SERT$^{+/-}$ rats choose the empty arms less often than SERT$^{+/+}$ rats as the rat IGT progresses. Shown is the mean proportion (±S.E.M.) of empty arm visits by SERT$^{+/+}$, SERT$^{+/-}$ and SERT$^{-/-}$ rats per block of 10 trials. *SERT$^{-/-}$ and SERT$^{+/-}$ rats different from SERT$^{+/+}$ rats (see text).

initial salience of disadvantageous cards promoted the establishment of conditioned responses directed to these disadvantageous choice options. In support, 5-HT affects the acquisition of fear conditioning (Burghardt et al., 2004). As the task progresses, a change in behavior is required, from exploration of the available options to the option with the best long-term outcome. The functional uncoupling between the rostral ACC (rACC) and the amygdala as reported in s-allele carriers is hypothesized to impair cortical control over amygdala function (Pezawas et al., 2005). It is possible that due to this rACC–amygdala functional uncoupling s/s subjects are not able to make this change, not able to use choice outcome (e.g. money loss) to guide the next choice, and persist with the choice that they acquired during the first phase of the task. Extinction of conditioned behaviors is based on this top–down feedback control system (Bishop, 2007). Findings that fear conditioning extinction recall is increased in SERT$^{-/-}$ mice (Wellman et al., 2007), extinction of an object operant task is retarded in monkeys carrying the s-allele (Izquierdo et al., 2007), and extinction of cocaine self-administration is delayed in SERT$^{-/-}$ rats (Homberg et al., unpublished observations), support the hypothesis that the s/s and SERT$^{-/-}$ genotypes in humans and rats, respectively, are associated with impaired top–down control. In other words, assuming that the emotional impact of earning money in humans, and of palatable sucrose pellets in hungry rats, are mediated by the amygdala, impaired top–down control over the amygdala will allow these emotional impacts to hijack the cognitive resources of the PFC that are needed for goal-directed behavior (Bechara, 2005).

Alternative explanations could involve genotype differences in punishment sensitivity, insofar 5-HT particularly signals adversity (Deakin and Graeff, 1991). As no 5-HTTLPR-related genotype differences were found in an IGT-version that is sensitive to high punishment (Must et al., 2007), this does not seem to be a plausible explanation. Further, we have shown previously that SERT$^{-/-}$ rats show improved inhibitory control (reduced premature responding) in the five-choice serial reaction time task (5-CSRTT; Homberg et al., 2007b), raising the possibility that the increased long-term choices in the rodent version of the IGT in SERT$^{-/-}$ rats reflects increased behavioral inhibition. However, premature responding is not a factor that is involved in the performance of the IGT. Finally, it is also not likely that the SERT genotype effects on IGT performance relate to differences in behavioral flexibility or switching per se, as no genotype differences between SERT$^{+/+}$, SERT$^{+/-}$ and SERT$^{-/-}$ rats were observed in a reversal learning task (Homberg et al., 2007b).

Poor decision-making is a symptom of several neuropsychiatric diseases that are related to disturbed 5-HT homeostasis, such as depression, psychostimulant dependence, schizophrenia, obsessive compulsive disorders, and obesity (Brand et al., 2006). Fully consistent with this notion, we show that s/s carriers of the 5-HTTLPR, for which numerous linkage studies have reported an association with these neuropsychiatric diseases (Murphy et al., 2004), perform poorly in the IGT. Two recent studies similarly showed that in depressive patients (Must et al., 2007) and in suicide attempters (Jollant et al., 2007) the IGT performance of s/s subjects was weaker than of l/l and l/s subjects. We extend these observations to healthy subjects.

An important limitation of our study is that the rat–human comparison is based on behavioral observations, and not on direct CNS comparisons. We are currently undertaking neuroimaging studies to bridge the gap between the species. Furthermore, we only studied female subjects, because female human subjects and female rats tend to choose more often long-term disadvantageous options in the IGT than male counterparts (Reavis and Overman, 2001; Van den Bos et al., 2006b, 2007; but see Overman, 2004), leaving only limited opportunity to detect any improvements in IGT performance. Interestingly, in a small pilot study SERT$^{+/+}$ and SERT$^{-/-}$ male rats performed the task equally well. This either suggests indeed that improvements are difficult to observe, at least in rats, or that gender-specific effects of genotype-dependent SERT dosage are present. Therefore, in future studies male subjects will be included as well. A third issue to address is that SERT$^{+/-}$ rats performed the IGT worse than SERT$^{+/+}$ and SERT$^{-/-}$ rats during the first phase of the task. This finding was somewhat unexpected as SERT$^{+/-}$ rats did not behave as intermediate between the two extremes. Although in other studies we also observed unexpected phenotypes of SERT$^{+/-}$ animals, we have no explanation for this phenomenon. Important, however, is that the extremes do not show differences during the first 60 trials. Another caveat could be that the recruited human subjects were of Dutch ancestry, which may have caused a bias in our data. It is therefore relevant to extend our findings to other ethnic populations. Finally, the human sample size is relatively small, as we used a population-based design. Notwithstanding, we obtained a significant effect, suggesting that the sample size was large enough to study the impact of 5-HTTLPR genotypes on decision-making.

Taken together, we show that in both human and rat SERT dosage affects long-term decision-making, thereby substantiating an important modulatory role of the serotonergic system in decision-making, which possibly involves a cortical top–down control mechanism. Although the 5-HTTLPR is one of the most widely studied genetic polymorphisms in psychiatry, interpretation of findings is hampered by the inconclusive data regarding 5-HT neurotransmission and/or neurotrophic processes. As such, comparing SERT$^{-/-}$ and SERT$^{+/-}$ rodent behavior with 5-HTTLPR phenotypes on comparable read-outs may be an approach to address this issue. Hence, our findings may open new lines of research to establish whether the SERT knockout models are valuable translational models for understanding mechanisms

underlying 5-HTTLPR-related phenotypes in healthy subjects and patients.

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