Serotonin transporter knockout rats show improved strategy set-shifting and reduced latent inhibition

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Behavioral flexibility is a cognitive process depending on prefrontal areas allowing adaptive responses to environmental changes. Serotonin transporter knockout (5-HTT<sup>-/-</sup>) rodents show improved reversal learning in addition to orbitofrontal cortex changes. Another form of behavioral flexibility, extradimensional strategy set-shifting (EDSS), heavily depends on the medial prefrontal cortex. This region shows functional changes in 5-HTT<sup>-/-</sup> rodents as well. Here we subjected 5-HTT<sup>-/-</sup> rats and their wild-type counterparts to an EDSS paradigm and a supplementary latent inhibition task. Results indicate that 5-HTT<sup>-/-</sup> rats also show improved EDSS, and indicate that reduced latent inhibition may contribute as an underlying mechanism.

[Supplemental material is available for this article.]
stimulus lights above the levers, which were extinguished after the rat responded on the inserted lever. Animals had to achieve a criterion of less than five omissions over a total of 90 trials and received at least five sessions of lever insertion training. When criterion was achieved, animals proceeded to the “side bias test” in which the potential side bias of animals was determined. During this test, trials were given in trial blocks (eight in total), in which rats had to press both levers in subsequent trials, after which a new trial block commenced. Thus, trials started with both levers inserted in the chamber and in the very first trial, lever pressing on either lever resulted in a sucrose pellet delivery. For reinforcer delivery in subsequent trials, rats had to press the lever opposite the one chosen at first. In case the same lever was pressed, no pellet reward was delivered and the house light was extinguished. This continued until this rat chose the lever opposite to the one chosen at first. The side bias was determined by considering the initial side which the rat pressed during the trial blocks and the total presses on one side during the whole session. If the total number of presses on either the left or right side was comparable to the number of presses on the other lever, the side that the rat initially pressed the most was its side bias. However, if the total number of presses on one side was disproportionate (i.e., >2:1) to the number of presses on the other side, the side with the most presses was considered the rat’s biased side. After side bias was determined, rats subsequently learned during the “strategy training” phase to use an egocentric strategy (e.g., always press the left lever, irrespective of the location of the illuminated stimulus light) for food reward. The lever opposite the rat’s preferred lever (as determined during the side bias test) was reinforced during this training. Rats continued to receive trials until either (1) at least 30 trials were completed and the criterion of eight consecutive correct responses was achieved or (2) after 120 trials, whichever came first. Trials in which the animal did not respond (i.e., omission trials) were not included in the trials to criterion measure. If rats did not succeed to reach the criterion measure within one session, the animal would receive a subsequent session of response training on the following day. After reaching criterion, rats proceeded with the subsequent “strategy set-shift” phase. Here, animals were required to cease the use of the formerly learned egocentric strategy and to shift toward the use of a visual stimulus-based strategy (i.e., always press the lever with the stimulus light illuminated above it, irrespective of left or right position). Rats continued to receive trials until either (1) a criterion performance of eight consecutive correct responses was achieved or (2) after 120 trials were given. Again, if a rat did not achieve criterion during one session, the animal received a subsequent session on the following day. The data of the strategy set-shift phase was additionally analyzed per block of 16 trials for three types of errors: perseverative errors, regressive errors, and never reinforced errors. Perseverative errors were scored when a rat pressed the lever without an illuminated stimulus light above it on the side that was initially reinforced during training. With regard to perseverative errors, if a rat pressed less than six perseverative errors in a block of 16 trials, the consecutive errors of this type were described as perseverative errors. Finally, never-reinforced errors were scored when a rat pressed the incorrect lever on trials using a strategy that was never reinforced during training or shift. Regressive and never-reinforced errors were used as an index of the animals’ ability to maintain and acquire a new strategy, respectively (Floresco et al. 2008).

With regard to the results of the initial strategy training phase, a One-Way ANOVA was performed using the trial-to-criterion data as dependent variable, and genotype as factor. This analysis revealed that 5-HTT+/+ rats were not different from 5-HTT−/− rats in the number of trials needed to reach the criterion of eight consecutive correct trials (F(1,13) = 0.752, not significant [NS]) on performance (see Fig. 1A, left). During the subsequent strategy set-shifting phase, animals had to shift from the use of an egocentric strategy (as learned during the training phase) toward a strategy that depended on the use of visual stimuli in order to obtain food reward. A One-Way ANOVA indicated that 5-HTT−/− rats needed considerably fewer trials to reach criterion than 5-HTT+/+ rats (F(1,13) = 9.054, P < 0.05) (see Fig. 1A, right). This was associated with a decreased amount of perseverative (F(1,13) = 5.262, P < 0.05), but not regressive (F(1,13) = 0.108, NS) or never-reinforced (F(1,13) = 1.972, NS) errors in 5-HTT−/− rats (see Fig. 1B). An additionally performed visual discrimination test indicated that the genotype differences in strategy set-shifting were not due to differences visual stimulus discrimination (see Supplemental Material).

The decrease in perseverative errors in 5-HTT−/− rats relative to 5-HTT+/+ animals can be attributed to two different processes. It may be based on enhanced “unlearning” of the previous egocentric strategy, but can also reflect enhanced associative stimulus-reward learning. The former possibility is not very likely in view of the fact that the two genotypes did not differ in the number of never-reinforced errors, implicating that both groups were equally likely to try an entirely novel strategy. With respect to the latter possibility, during the initial training phase, the visual stimulus was irrelevant. In other words, this training can be conceptualized as “learned irrelevance” training, which the rats had to overcome after the shift. This exposure-induced learned irrelevance and associated decrement in learning a stimulus-reward association is similar to the phenomenon of latent inhibition (Lubow 1973). To further investigate the validity of a learned irrelevance account, an additional batch of animals was tested in a latent inhibition task, which used an auditory stimulus as conditioned stimulus (CS) and sucrose pellets as positive reinforcer. A total of four groups (pre 5-HTT+/+ [n = 8], no-pre 5-HTT+/+ [n = 7], pre 5-HTT−/− [n = 8], no-pre 5-HTT−/− [n = 8]) of rats were tested in a previously described setup (Nonkes et al. 2010). All animals were food deprived 2 h before experimental testing and received two sessions of magazine training to ensure frequent visits to the food magazine (15 pellet deliveries on a random interval schedule of 4 min ± 25%). Subsequently, pre 5-HTT+/+ and pre 5-HTT−/− rats received pre-exposure consisting of six sessions, during which rats were exposed 10 times to a 3-kHz auditory CS (76 dB, 30 sec) at a 4-min intertrial interval (±25%). Rats of the no-pre 5-HTT−/− and no-pre 5-HTT−/− groups were placed in the Skinner boxes for an equal amount of time, but without the tone presentations. Successively, all animals received eight conditioning sessions, during which the CS was presented 10 times at a 4-min intertrial interval (±25%). The end of each CS coincided with the delivery of a sucrose pellet to the food magazine. The number of visits to the food magazine was measured during
Conditioning was indexed as the mean (or not received (no-pre) nonreinforced pre-exposures to the CS. Used as CS across conditioning sessions in the final phase of the latent in-
sent the increase in conditioned responding to the visual cue that was
have been reported in 5-HTT
2008), and latent inhibition paradigms (George et al. 2010). As an-
(strategy) set-shifting (Ragozzino et al. 1999; Floresco et al.
ibility. Specifically, the orbitofrontal cortex (OFC) is implicated in
during which latent inhibition effects can be expected—but not
pre-exposure, whereas 5-HTT−/− rats showed a significant reduction in conditioning compared with their no-pre-group counterparts.
In summary, we show here that 5-HTT+/− rats outperformed
inhibition effect after pre-exposure, whereas 5-HTT−/− rats failed
do so. Therefore, it is conceivable that reduced latent inhibi-
tion/learned irrelevance effects contributed to the improved set-
hiﬁlating performance of 5-HTT−/− rats. The observation that
5-HTT−/− rats showed a reduced number of perseverative errors—which occur during the early phase of the shift, the period
within which latent inhibition effects can be expected—but not
regressive or never-reinforced errors supports this notion.

The prefrontal cortical areas are essential for behavioral ﬂex-
ibility. Speciﬁcally, the orbitofrontal cortex (OFC) is implicated in
reversal learning (McAlonan and Brown 2003; Ghods-Sharif et al.
2008), the medial prefrontal cortex (mPFC) in extradimensional
(strategy) set-shifting (Ragazzino et al. 1999; Floresco et al.
2008), and latent inhibition paradigms (George et al. 2010). As an-
tomical and task-related functional changes in these regions
have been reported in 5-HTT−/− rodents (Wellman et al. 2007;
Nonkes et al. 2010; Pang et al. 2011), the observed genotype diffe-
ences in reversal learning (Nonkes et al. 2011) and the EDSS and
latent inhibition paradigms (present study) might relate to altered
OFC and mPFC function. In line, we speciﬁcally observed a geno-
type effect on mPFC-dependent perseverative errors, but not re-
gressive or never-reinforced errors, which are thought to be
more striatum-dependent (Floresco et al. 2009).

One limitation of the present study is that we did not incor-
porate a retention test after the set-shift training phase to deter-
mine whether a consolidation and/or retention deﬁcit may have
contributed to the improved 5-HTT−/− rats’ set-shifting performance. However, previous experiments performed with
5-HTT−/− rats did not provide any evidence for the notion that
5-HTT−/− rats exhibit a consolidation/retention deﬁcit (e.g.,
Nonkes et al. 2010, 2011). Hence, it is unlikely that this contribut-
ed to the observed improved set-shift performance. Another lim-
itation is that we tested the animals for strategy set-shifting in a
noncounterbalanced design. Thus, rats were tested only in one
shift-direction (egocentric stimulus -> visual stimulus). As such,
remains to be clariﬁed whether true attentional set-shifting is af-
fected by 5-HTT genotype or discrimination learning (Baxter and
Gaffan 2007). Nevertheless, the results from the performed latent
inhibition task suggest that alterations in 5-HTT levels do affect at-
tentional processes.

Our study complements earlier ones, indicating that 5-HTT
‘genetic variance affects, next to emotionality, aspects of cognitive
functioning (Homberg and Lesch 2011). As such, 5-HTT genetic
down-regulation may make an individual more vulnerable for
developing anxiety- and depression-like symptoms (Caspi et al.
2010). However, the same genetically linked trait may contribute
to behavioral ﬂexibility, potentially through a hyper-reactive
mPFC (Heinz et al. 2005) and OFC (Kalin et al. 2008). 5-HTT ge-
netic variance may therefore be an important mediator for how
an individual interacts with its environment, for better and for
worse.

Acknowledgments
We thank A. Afraisiab-Middelman, H. Krijnen, and S. Hermeling
for their help with the animal care. This work was funded by
The Netherlands Organization for Scientiﬁc Research (NWO),
grant #64140003, awarded to J.H.R.M. NWO had no further role
in the design of the study; in the collection, analysis, and interpre-
tation of data; in the writing of the report; or in the decision to
submit the paper for publication.

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Set-shifting in serotonin transporter knockout rat


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Access the most recent version at doi:10.1101/lm.025908.112

Supplemental Material
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