Differential Effects of Serotonin Transporter Genotype on Anxiety-Like Behavior and Cognitive Judgment Bias in Mice

Viktoria Krakenberg1,2*, Vanessa Tabea von Kortzfleisch1,2, Sylvia Kaiser1,2, Norbert Sachser1,2 and S. Helene Richter1,2

1 Department of Behavioural Biology, University of Münster, Münster, Germany, 2 Otto Creutzfeldt Center for Cognitive and Behavioral Neuroscience, University of Münster, Münster, Germany

In humans, the short allele of a common polymorphism in the serotonin transporter (5-HTT) gene is associated with a higher risk to develop depression and anxiety disorders. Furthermore, individuals carrying this allele are characterized by negative judgment biases, as they tend to interpret ambiguous information in a more pessimistic way. 5-HTT knockout mice, lacking the 5-HTT gene either homo- or heterozygously, provide a widely used model organism for the study of symptoms related to human anxiety disorders. In the present study, we aimed to prove the anxiety-like phenotype of the 5-HTT mouse model, and to investigate whether 5-HTT genotype also causes differences in judgment bias. While our results confirm that homozygous 5-HTT knockout mice display highest levels of anxiety-like behavior, it was decreased in heterozygous mice. Against our expectations, we did not detect differences in the animals’ judgment bias. These results indicate that at least in mice the association between 5-HTT genotype and judgment bias is not straightforward and that other factors, including multiple genes as well as environmental influences, are implicated in the modulation of judgment biases. More research is needed to gain further insights into their function as potential endophenotypes for psychopathology.

Keywords: anxiety, serotonin transporter, 5-HTT, cognitive judgment bias, pessimism, endophenotype, mice

INTRODUCTION

Anxiety disorders are among the most prevalent psychiatric disorders, severely compromising an individual’s quality of life (Bandelow and Michaelis, 2015). Environmental and genetic factors as well as their interaction are regarded as contributing causes (Caspí and Moffitt, 2006; Caspi et al., 2010). Among the genetic risk factors, the serotonin transporter (5-HTT) gene constitutes an important candidate, with a common polymorphism in the 5-HTT-linked polymorphic region, appearing in form of either a short (s) or a long (l) allele (Lesch et al., 1996; Gross and Hen, 2004; Canli and Lesch, 2007). Carrying the s-allele has been linked to anxiety-related personality traits and is associated with an increased risk to develop anxiety disorders and depression, especially following adverse life events (Lesch et al., 1996; Mazzanti et al., 1998; Greenberg et al., 2000; Caspi et al., 2003, 2010).

A shared characteristic of individuals with anxiety disorders is that they selectively process information in a way favoring negative emotional valence, a phenomenon also referred to as cognitive bias (Mathews and MacLeod, 1994, 2005). Such negative cognitive biases comprise attention (e.g., enhanced attention toward threatening stimuli), memory (e.g., the predominant...
recall of negative memories), and judgment biases (e.g., pessimistic interpretations of ambiguous information) (Mathews and MacLeod, 2005). Evidence suggests a genetic component of such biases, with the 5-HTT polymorphism being implicated in their development and maintenance (Fox et al., 2009; Fox and Standage, 2012). For instance, 5-HTT s-allele carriers were found to display enhanced attention toward and no active avoidance of negative information (Beevers et al., 2007; Fox et al., 2009), and to interpret ambiguous homophones more pessimistically compared to l-allele carriers (Fox and Standage, 2012). Therefore, cognitive biases may provide important endophenotypes for anxiety disorders (Fox et al., 2009; Fox and Standage, 2012).

Based on the human cognitive bias concept, the development of the first cognitive bias test for rats (Harding et al., 2004) paved the way for the assessment of judgment biases in a variety of animal species (e.g., Kloke et al., 2014; Bethell, 2015; Hintze et al., 2018; Jones et al., 2018; Krakenberg et al., 2019). In animals, similar to humans, negative judgment biases often co-occur with higher levels of anxiety-like and depression-related behavior, implying that the underlying mechanisms might be shared among species (but see for instance Bethell and Koyama, 2015). This was mainly shown on the basis of studies using emotion-manipulating treatments to induce specific emotional states. For instance, isolation stress in chicken (Salmeto et al., 2011), as well as changes in light intensity or chronic stress exposure in rats (Burman et al., 2009; Rygula et al., 2013) were reflected in the animals' judgment bias. Likewise, also approaches using genetic animal models without previous induction of anxiety- or depression-like states revealed mood-congruent differences in judgment bias (e.g., congenitally helpless rat strains) (Enkel et al., 2010; Richter et al., 2012; Kloke et al., 2014).

As mice are the predominantly used mammalian animal model, the aim of the present study was to investigate, whether cognitive biases may constitute potential endophenotypes for anxiety disorders in this species. Therefore, we applied the 5-HTT knock out mouse model (Bengel et al., 1998). We studied male wild type (+/+; n = 15), heterozygous (+/−; n = 14) and homozygous (−/−; n = 11) knockout mice (deviations from sample sizes due to technical problems during behavioral testing are indicated in the results section). As six mice (+/+: n = 1; +/−: n = 2; −/−: n = 3) displayed a continuous lack of improvement in training performance they were excluded from the study and are not included in the above mentioned sample sizes. The animals originated from the internal breeding stock of the Department of Behavioral Biology at the University of Münster, Germany. The original heterozygous breeding pairs were provided by the Department of Molecular Psychiatry at the University of Würzburg, Germany. For genotyping, genomic DNA was extracted from ear tissue and amplified by PCR. Genotypes were identified by agarose gel electrophoresis of DNA fragments with a lengths of 225 bp (5-HTT +/−), 272 bp (5-HTT −/−) or both (5-HTT +/−). After weaning, mice were housed in groups of 2–5 animals per cage (Makronen cages type III, 38 × 23 × 15 cm3). At the age of approximately 9 weeks, they were transferred to single cages to avoid any escalated aggression. For four mice (+/+: n = 1; +/−: n = 2; −/−: n = 1), single housing had to be initiated before that age due to incidences of escalated fighting. Cages contained wood shavings as bedding material (Allspan, Höveler GmbH & Co. KG, Langenfeld, Germany), a paper towel, a wooden stick and a semi-transparent red plastic house (11.1 × 11.1 × 5.5 cm3, Tecniplast Deutschland GmbH, Hohenpeissenberg, Germany). Housing rooms were maintained at a reversed dark/light cycle with lights off at 10.00 a.m., a temperature of approximately 22°C, and a relative humidity of about 50%. Mice were provided with water and food (Altromin 1314; Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) ad libitum, except for the time during touchscreen training and cognitive bias testing, when they were food restricted to 90–95% of their ad libitum feeding weights in order to enhance their motivation to work for food rewards. During the food restriction period, the animals’ body weights remained constant. For details regarding the animals’ body weights see Supplementary Figure S4.

Ethics Statement

All procedures complied with the regulations covering animal experimentation within Germany (Animal Welfare Act) and the
EU (European Communities Council DIRECTIVE 2010/63/EU) and were approved by the local (Gesundheits- und Veterinäramt Münster, Nordrhein-Westfalen) and federal authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen “LANUV NRW,” reference number 84-02.04.2015.A441).

Experimental Design
The aims of this study were (I) to assess CJB as a potential cognitive endophenotype for anxiety disorders in mice varying in their serotonin transporter genotype (5-HTT+/+, +/−, and −/−), and (II) to confirm the anxiety-like phenotype of the mouse model applied. The experiment comprised four phases: a handling phase, a training phase, a CJB test phase, and a behavioral test phase (see Figure 1). Mice entered the experimental phase batch-wise, with each batch comprising animals of at least two different genotypes. The handling phase started at the age of 10 ± 1 weeks. During this phase, mice were accustomed to cup handling, a method suggested to reduce anxiety in mice (Hurst and West, 2010). The individual weights of all mice were monitored on each experimental day until the start of the behavioral test phase. During the training phase, which started at 12 ± 1 weeks, mice had to acquire a discrimination task. As training success was determined on an individual basis, training durations differed among animals (for a comparison of training durations between groups see Supplementary Figure S1). After successful discrimination training, mice proceeded to the CJB test phase at an age of 30 ± 12 weeks. At the end of this phase, they were fed ad libitum diet again. 4 ± 1 weeks later, mice entered a battery of behavioral tests comprising the Elevated plus maze test (EPM), Dark light test (DL), Open field test (OF), and Free exploration test (FE), in order to assess their anxiety-like and exploratory behavior. The experimenter was blind to the genotypes of all mice during the experimental phase.

Cognitive Judgment Bias Test
Cognitive judgment bias was assessed using an automated touchscreen system for mice (Bussey-Saksida Mouse Touch Screens, Chalmers University of Technology, Malmo, Sweden). The touchscreen system has been described previously (Richter et al., 2014; Krakenberg et al., 2019). Briefly, it comprised four independent trapezoid shaped chambers (base area: 260 cm², height: 20 cm), each equipped with a tone generator, a house light, an infrared sensitive touchscreen at the front (24.5 × 18.5 cm²) and a reward magazine (2 × 2 × 2 cm³) with a well for reward collection at the rear end. Rewards consisted of servings of sweet condensed milk, diluted 1:4 in tap water (in the following referred to as “SCM”). The touchscreen was covered by a black Perspex mask with three adjoining windows (7 × 7 cm²) providing access to the screen. On the central window (cue presentation field), cues in form of white bars (6 × 1 cm²) could be displayed at different positions. Mice had to nose poke a gray cross (width: 6 cm, height: 6 cm) on either the left or right window (touch fields) in response to these cues.

Apparatus
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Paradigm
The paradigm was based on a discrimination task, in which mice had to learn to distinguish between a positive and a negative condition, signaled either by a bar displayed at the bottom (positive condition, P), or at the top of the cue presentation field (negative condition, N). During the positive condition, one touch field was associated with a large (12 µl SCM), the other one with a small reward (4 µl SCM). Mice had to learn to touch the high-rewarded field in this condition. During the negative condition, however, this previously high-rewarded touch field was associated with a mild “punishment” (5 s time out and house light on). The opposite field was again associated with a small reward (4 µl SCM). Mice had to learn to touch the small-rewarded field in this condition. While the cues signaling the positive or the negative condition, respectively, were the same
for all mice, the association of cue (positive/negative) and correct touch field (left/right) was counterbalanced across animals of the three treatment groups. As soon as a mouse had acquired the task, it was tested in the CJB test. During the test, each animal was presented with cues displayed at three intermediate positions: middle (M), near positive (NP), and near negative (NN). The M condition was regarded as most ambiguous. The NP and NN conditions were also regarded as ambiguous, however, to a lesser degree. Responses to these ambiguous cues served as a measure of the animals’ judgment bias (Figure 1).

**Procedure**

Touchscreen training was carried out on a daily basis with exceptional breaks of about 3 days. For each touchscreen session, a mouse was taken out of its home cage, weighed, and transported to the adjacent room within a red, semi-transparent box (21 × 21 × 15 cm$^3$). Consequently, the mouse was placed into a touchscreen chamber, the respective session was started and ended after a predetermined duration or as soon as a maximum number of trials was completed, depending on the respective training step. After completion of each touchscreen session, mice were returned to their home cages and received their daily food ration.

At the beginning of the training phase, mice acquired basic skills to operate within the chambers, such as touching for a reward, initiating trials by nose poking into the reward magazine and becoming accustomed to the mild “punishment” upon incorrect touches. This phase is commonly referred to as pre-training and followed the protocol described previously (Krakenberg et al., 2019).

Subsequently, mice entered the actual discrimination training. It was conducted according to our previously established protocol with some modifications (for details see following section and Krakenberg et al., 2019). The aim of this phase was the reliable discrimination between the positive and the negative condition by the mice. An overview of all discrimination training steps with final criteria for progression to the respective next training step is given in Table 1. The time of each training session was limited to 30 min.

Divergent from our previous protocol, we refined the first discrimination training step, which we hereafter refer to as discrimination training 1. This step had to be implemented during the ongoing experimental phase, as the direct transition to the consecutive step (discrimination training 2) resulted in substantial learning difficulties of the mice. During discrimination training 1, mice were presented with 25 positive and 25 negative trials per session in a pseudo-randomized order. In response to each cue, only touches on the correct touch field resulted in a criterion (large reward during positive condition, small reward during negative condition), while the respective incorrect touch field was inactivated so that touches on this field did not result in any outcome at all. A reasonable criterion for the progression to the next step turned out to be the completion of at least five sessions, with the last two sessions being completed in less than 20 min. This criterion was developed during the course of the experiment and was thus not applied to all animals equally. If animals did not show an improvement of learning performance during later training steps, they were returned to discrimination training 1.

Subsequently, mice proceeded to the regular training schedule, comprising discrimination trainings 2, 3, and 4. Briefly, they were confronted with balanced numbers of positive and negative trials in a pseudo-randomized order. Trial numbers increased from 20 (discrimination training 2) to 50 (discrimination trainings 3 and 4). Until discrimination training 3, incorrect touches were followed by correction trials, i.e., the same trial was repeatedly presented until a correct touch was executed. As soon as a response accuracy of 80% was reached, this was confirmed once more without correction trials in discrimination training 4.

We furthermore added another training step to the previous protocol, discrimination training 5, during which mice were confronted with so-called “pseudo-probe trials.” These were pseudo-randomly chosen positive or negative training trials (3 × P and 3 × N per training session). Positive pseudo-probe trials remained unrewarded, regardless of whether mice touched correctly or incorrectly. Negative pseudo-probe trials remained unrewarded when touching correctly, but also unpunished when touching incorrectly. Firstly, this way of partial reinforcement was introduced in order to accustom the animals to the probe trials during the following test phase, which were neither rewarded nor punished. Secondly, these pseudo-probe trials were applied to prevent mice from learning the outcome of the ambiguous trials during testing (Roelofs et al., 2016). Per session, six pseudo-probe trials were presented and interspersed with 44 regular training trials. Mice had to stay in discrimination training 5 for a minimum of three sessions, and could proceed after the last two sessions were completed with at least 80% correct responses.

Once mice had successfully completed the discrimination task, they were tested for their CBJ. Subjects underwent five test sessions at intervals of approximately 24 h with maximally one gap day in between. For this purpose, mice were first weighed and then carried to the adjacent room in the red transport box, where the touchscreen session was started. Each test session comprised 50 trials. Per session, six probe trials (2 × NP, 2 × M,

### Table 1 | Discrimination training steps.

| Discrimination training | Max. number of trials | Criterion | Correction trials |
|-------------------------|-----------------------|-----------|-------------------|
| 1                       | 50                    | ≥5 sessions, completion of 50 trials in ≥20 min. in last two sessions | no |
| 2                       | 20                    | 80% correct responses in two consecutive sessions | yes |
| 3                       | 50                    | 80% correct responses in two consecutive sessions | yes |
| 4                       | 50                    | 80% correct responses in one session | no |
| 5                       | 50                    | ≥3 sessions, 80% correct responses in last two sessions | no |

Training consisted of 5 steps. Training sessions ended after 30 min, unless the maximum number of trials was reached before that time. Upon incorrect touches, correction trials (i.e., repeated presentation of the same trial until a correct touch) were presented (not included in the total trial count).

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Two opposing closed and open arms (30 × 30 cm² on the same day was randomized. Instead, upon touching, cue as well as touch symbols disappeared and the animal could initiate the next trial. At the end of the test phase, each ambiguous cue had been presented 10 times, each trained cue 110 times, amounting to a total number of 250 trials that were completed during the test phase.

**Behavioral Measures**

The responses to the ambiguous cues during test sessions were taken as a measure of the animals’ CBJ. “Optimistic” choices were defined as touches according to the positive condition, “pessimistic” choices as touches according to the negative condition. Out of these choices in response to each condition, a score (choice score) was calculated as follows:

\[
\text{Choice score} = \frac{N \text{ choices } (“\text{optimistic}“) - N \text{ choices } (“\text{pessimistic}“)}{N \text{ choices } (“\text{optimistic}“ + “\text{pessimistic}“)}
\]

Scores could range from −1 to +1. Higher choice scores indicate a higher proportion of “optimistic” choices, i.e., a more positive CBJ as compared to lower choice scores. Please note that the term “choice score” was chosen for the sake of intuitiveness. It is not supposed to imply that positive scores can be regarded as “optimistic” and negative scores as “pessimistic” per se. Scores should always be interpreted in relation to each other.

**Anxiety-Like and Exploratory Behavior**

Mice were subjected to a battery of four behavioral tests, assessing state as well as trait anxiety and exploratory locomotion. State anxiety, defined as the anxiety an individual experiences at a specific moment in time and in response to an anxiogenic stimulus, was assessed in our study by conducting the EPM test, DL test, and OF test (Lister, 1990). Trait anxiety, on the contrary, is considered to be an enduring feature of an individual and was assessed using the FE test (Lister, 1990; Griebel et al., 1993; Ramos and Mormède, 1998). The test battery was scheduled 4 ± 1 weeks after the cognitive bias test at an age of 35 ± 12 (EPM, DL, and OF) and 36 ± 12 weeks (FE). To avoid any influence of food restriction on test outcomes, mice were fed ad libitum diet after CBJ testing again. Tests were carried out at intervals of at least 48 h and at the beginning of the dark phase between 10.15 and 12.00 a.m. Before each test, the respective apparatus was cleaned with 70% ethanol. Mice were carried to the testing room within the red transport box for the EPM, DL, and OF and within their home cage for the FE. All tests were recorded with a camera (Logitech Webcam Pro 9000) and the video tracking software ANY-maze (version 4.99M, release: 2014, Stoelting Co., Wood Dale, IL, United States). Immediately after each test was started, the experimenter left the room quietly. The order of mice tested on the same day was randomized.

**Elevated Plus Maze Test (EPM)**

The apparatus of the EPM (Pellow et al., 1985; Lister, 1987, 1990) consisted of a plus-shaped maze, elevated 50 cm above the floor. Two opposing closed and open arms (30 × 5 cm²), respectively, extended from a central square area (5 × 5 cm²). Closed arms were surrounded by gray, 20 cm high wooden walls, open arms by a 4 mm high border to prevent the mice from falling down. The floor of the maze was covered by a gray PVC inlay. Illumination intensity in the central square was 25 lux. Mice stayed in the transport box for 1 min and were then placed on the central square of the EPM, always facing the same closed arm. They were allowed to explore the maze for 5 min. The relative time spent on the open arms, the relative number of entries into the open arms and the distance traveled on the open arms were taken as measures of the animals’ anxiety-like behavior. The sum of entries made into the open and closed arms of the apparatus was taken as a measure of their exploratory locomotion.

**Dark Light Test (DL)**

The apparatus of the DL test (Crawley and Goodwin, 1980) consisted of a modified Makrolon cage type III. The cage was divided into a light and a dark compartment by a gray PVC wall. The wall had an opening (10 × 4 cm) which could be closed with a sliding door. The dark compartment measured one third of the cage size, its walls were painted black and it could be closed with a gray PVC lid. The light compartment was illuminated with an intensity of 40 lux. Mice were placed into the dark compartment with the sliding door closed. After 1 min, the sliding door was opened and the mouse could freely explore the apparatus for 5 min. The time spent in and the latency to enter the light compartment were used as measures of the animals’ anxiety-like behavior, the number of entries made into the light compartment as a measure of their exploratory locomotion.

**Open Field Test (OF)**

The apparatus of the OF test (Archer, 1973; Treit and Fundytus, 1989) consisted of a white coated plywood box, comprising a square arena (80 × 80 cm²) framed by 42 cm high walls. Light intensity in the center of the arena was set to 35 lux. After arrival in the testing room, mice stayed in the transport box for 1 min before testing started. Mice were then placed into the arena, always facing the same corner, and were allowed to explore the apparatus for 5 min. Measures of anxiety-like behavior were the time spent in as well as the number of entries made into the center of the OF, defined as the area being located at least 20 cm distant from the walls. The total distance traveled was taken as a measure of exploratory locomotion.

**Free Exploration Test (FE)**

The apparatus of the FE test (Griebel et al., 1993) consisted of a white coated plywood box, comprising a square arena (60 × 60 cm²) framed by 35 cm high walls. In one wall there was an opening measuring 11 × 15 cm². Light intensity in the center of the arena was set to 35 lux. The home cage of the animals was connected to the arena via a Plexiglas tunnel attached to the opening. The animals were transported into the testing room in their home cages, which were covered with black cloth to protect them from light. Mice were placed into the red transport box for 1 min after arrival in the testing room, before they were transferred to the home cage again. The home cage was equipped with a sliding door for this test, which was opened just before the
RESULTS

Cognitive Judgment Bias

There was a significant main effect of condition in the cognitive bias test ($F_{(4,156)} = 501.725, p \leq 0.001, \eta^2_{p} = 0.93$; Figure 2). Post hoc comparisons showed significant differences in the choice scores between adjacent conditions ($p \leq 0.001$), except between P-NP and NN-N ($p \geq 0.8$). On a descriptive level, choice scores resulted in a response curve, decreasing from the positive to the negative condition. However, we neither detected a main effect of genotype ($F_{(2,83)} = 0.096, p = 0.909, \eta^2_{p} = 0.002$), nor a condition x genotype interaction ($F_{(8,156)} = 0.459, p = 0.883, \eta^2_{p} = 0.02$). For statistical details and individual response curves see Supplementary Figure S3 and Supplementary Table S1.

Anxiety-Like and Exploratory Behavior

Anxiety-like and exploratory behavior of the animals was assessed using the EPM, DL test, OF test, and FE test. While the animals’ behavior in the EPM, DL, and OF reflects their state anxiety, trait anxiety was assessed in the FE. An overview of statistical parameters of the analysis is given in Table 2. For graphs of all parameters assessed see Supplementary Figure S2.

Anxiety-Like Behavior

There was a significant main effect of genotype on anxiety-like behavior in the EPM, reflected by the time the mice spent on the open arms ($F_{(2,39)} = 38.562, p \leq 0.001$; Figure 3A), the entries they made into the open arms ($F_{(2,24)} = 8.404, p = 0.002$) and the distance they traveled on the open arms of the apparatus ($F_{(2,16)} = 25.384, p \leq 0.001$). Post hoc analysis showed that 5-HTT $+/-$ mice displayed the highest levels of anxiety-like behavior, with the least time spent on the open arms ($+/+ \text{ vs. } -/-: p \leq 0.001, +/+ \text{ vs. } -/-: p \leq 0.001$), the fewest entries made into these ($+/+ \text{ vs. } -/-: p = 0.023, +/+ \text{ vs. } -/-: p = 0.002$) and the shortest distances traveled there ($+/+ \text{ vs. } -/-: p = 0.012, +/+ \text{ vs. } -/-: p \leq 0.001$). Interestingly, levels of anxiety-like behavior were lowest in the 5-HTT $+/+$ group, indicated by significantly more time spent on the open arms ($+/+ \text{ vs. } +/+: p = 0.010$) and longer distances traveled there compared to 5-HTT $+/-$ animals ($+/+ \text{ vs. } +/+: p = 0.002$).

Furthermore, there was a main effect of genotype on anxiety-like behavior in the OF, indicated by the time the mice spent in the center ($F_{(2,36)} = 11.380, p \leq 0.001$; Figure 3B) and the number of entries they made into the center of the apparatus ($F_{(2,36)} = 14.710, p \leq 0.001$). Again, 5-HTT $-/-$ mice displayed highest levels of anxiety-like behavior, as they spent less time in the center ($+/+ \text{ vs. } -/-: p \leq 0.001, +/+ \text{ vs. } -/-: p \leq 0.001$) and entered it fewer times compared to both 5-HTT $+/+$ and 5-HTT $+/-$ mice ($+/+ \text{ vs. } -/-: p \leq 0.001, +/+ \text{ vs. } -/-: p \leq 0.001$).

Furthermore, there was a trend for a main effect of genotype on the time spent in the light compartment of the DL ($F_{(2,32)} = 2.859, p = 0.072$; Figure 3C). Post hoc comparisons revealed that by trend 5-HTT $-/-$ mice spent less time in start of the test and the animals could freely explore the arena for 15 min. The time spent in and the latency to enter the arena were used as measures of the animals’ anxiety-like behavior, the number of entries made into the arena as a measure of their exploratory locomotion.

Statistics

To check for the assumptions of parametric analysis, residuals were examined for heteroscedasticity and normal distribution graphically and using the Shapiro–Wilk normality test. If the assumptions were not met, data were transformed using square root (DL: entries into light compartment, FE: latency to enter arena) or logarithmic transformations (OF: time spent in center and entries into center). CBj test data were analyzed using a linear mixed-effects model (LMM) including “condition” as fixed within-subject factor, “genotype” as fixed between-subject factor and “age” as a random between-subject factor. Main effect of condition ($p \leq 0.001$). Sample sizes: $n+/+ = 15, n+/-/ = 14, n-/-/ = 11$. $P =$ positive, $NP =$ near positive, $M =$ middle, $NN =$ near negative, $N =$ negative.

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![Figure 2](http://www.frontiersin.org/) Cognitive judgment bias. Choice scores are given as means ± SEM. Statistics: LMM with “condition” as fixed within-subject factor, “genotype” as fixed between-subject factor and “age” as a random between-subject factor. Main effect of condition ($p \leq 0.001$). Sample sizes: $n+/+ = 15, n+/-/ = 14, n-/-/ = 11$. $P =$ positive, $NP =$ near positive, $M =$ middle, $NN =$ near negative, $N =$ negative.
the light compartment compared to 5-HTT +/- mice (+/+ vs. −/−: p = 0.073). However, we did not detect an effect of genotype on the latency to enter the light compartment (F(2,37) = 0.851, p = 0.435).

In the FE, the latency to enter the arena was affected by genotype (F(2,21) = 4.203, p = 0.029; Figure 3D), but we did not detect an effect of genotype on the time the animals spent there (F(2,36) = 1.013, p = 0.373). Again, higher levels of anxiety-like behavior were found in 5-HTT −/− mice, indicated by longer latencies until they entered the arena compared to 5-HTT +/- mice (+/+ vs. −/−: p = 0.023).

### DISCUSSION

In the present study, we aimed to investigate the role of judgment biases as potential endophenotypes for anxiety disorders in the 5-HTT mouse model. We therefore assessed the anxiety-like phenotype of mice varying in 5-HTT genotype in a battery of standardized tests, as well as their CBJ by...
using a touchscreen paradigm of potentially high translational value (Talpos and Steckler, 2013). While anxiety-like behavior was highest in 5-HTT −/− and lowest in 5-HTT +/+ mice, we did not detect genotype differences in the animals’ judgment bias.

**Anxiety-Like Behavior**

5-HTT −/− mice displayed lowest levels of exploratory locomotion and highest levels of anxiety-like behavior in the EPM, DL, and OF, reflecting the animals’ state anxiety. Anxiety-like behavior was also highest in these animals in the FE which
targets trait anxiety. These results are consistent with previous findings and prove the applicability of the 5-HTT mouse model for the study of symptoms related to human anxiety disorders (Holmes et al., 2003; Carroll et al., 2007; Heiming et al., 2009).

Unexpectedly, 5-HTT +/− mice showed lowest levels of anxiety-like behavior in the EPM compared to wild type and homozygous knockout mice. In contrast to this, the majority of previous studies report heterozygous mice to be characterized by a tendency to show higher levels of anxiety-like behavior than wild type mice, similar to homozygous knockouts, yet, this anxiety-like phenotype only emerged in response to experimentally induced adversity (Carola et al., 2008; Jansen et al., 2010; van den Hove et al., 2011). Based on these studies, we would have expected heterozygous mice to rather show similar levels of anxiety-like behavior compared to wild type mice, as we did not provide any experimental treatment. In humans, however, reduced 5-HTT function is not only considered to confer a higher vulnerability to adversity, but rather a higher susceptibility to environmental influences in general, including also those of positive valence. This phenomenon has been referred to as the differential susceptibility hypothesis (Belsky et al., 2009). S-allele carriers are thus considered to be more plastic, instead of simply more vulnerable to adversity (Belsky et al., 2009). The differential susceptibility hypothesis is supported by first findings in mice: Kästner et al. (2015) showed heterozygous 5-HTT knockout mice to be more susceptible to the positive experience of cohabitation with a female. In comparison to wild type controls, heterozygous mice displayed a less-anxiety-like phenotype (Kästner et al., 2015).

The anxiety-like phenotype of the heterozygous mice in the present study matches the results of Kästner et al. (2015). However, we did not intentionally provide any beneficial experimental treatment. The only experimental procedure the mice underwent before entering the behavioral test battery was the cognitive bias task, including an intensive training phase. This might be a hint for a potential influence of the judgment bias paradigm itself on the behavioral profile of 5-HTT mice. A study addressing the effects of daily exposure to touchscreen training on mice supports this suggestion: Mallien et al. (2016) found mice to display elevated corticosterone levels in anticipation of touchscreen training. Such a glucocorticoid response does not only follow aversive conditions, but can also occur under conditions generally assumed to be of positive valence, e.g., during sexual behavior or environmentally enriched housing conditions (Marashi et al., 2003; Koolhaas et al., 2011). In accordance, Mallien et al. (2016) interpret their findings as a potential indicator of an enrichment-like effect elicited by the training procedure. Assuming a potentially beneficial effect of touchscreen training, our results might be another hint for a differential susceptibility conferred by reduced 5-HTT function.

Cognitive Judgment Bias

There was a significant effect of condition in the CBJ test, a finding of particular interest in the light of the differences we found in the animals’ anxiety-like behavior. This contradicts our expectations based on previous studies, in humans as well as in mice. In humans, 5-HTT genotype has been associated with biases in attention as well as ambiguous cue interpretation (Bevers et al., 2007; Fox et al., 2009; Fox and Standage, 2012). Despite the many physiological and behavioral similarities between human s-allele carriers and 5-HTT knockout mice, however, human studies hardly allow controlling for the background genotype or environmental effects across the subjects’ life span (Holmes et al., 2003). This should be kept in mind when comparing results between human and mouse studies, particularly because the 5-HTT s-allele is considered to confer a higher susceptibility to environmental influences (Belsky et al., 2009). Moreover, most studies in humans focused on attention biases, while here we assessed judgment bias (e.g., Bevers et al., 2007; Fox et al., 2009). It would thus be of great interest to additionally assess different subtypes of cognitive biases (attention, memory biases) in mice in the future, as these might be affected differently by 5-HTT function. Yet, to date, the necessary paradigms for assessing attention or memory bias in mice are lacking.

In mice, Kloke et al. (2014) found a very first hint for a role of 5-HTT genotype in the modulation of judgment biases, as they report a trend for homozygous 5-HTT knockout mice to display a pessimistic-like judgment bias. However, this finding was based
Moffitt, 2006). More research is needed to gain further insights into the function of cognitive biases as potential endophenotypes for psychopathology.

**DATA AVAILABILITY STATEMENT**

The datasets generated for this study are available on request to the corresponding author.

**ETHICS STATEMENT**

All procedures were reviewed and approved by both the Gesundheits-und Veterinäramt Münster, Nordrhein-Westfalen, Germany and the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen, Germany.

**AUTHOR CONTRIBUTIONS**

SR, NS, and SK conceived the study. SR, NS, SK, and VK designed the experiments. VK carried out the experiments. VK and VvK conducted the statistical analysis of the data. SR and NS supervised the project. VK wrote the initial draft of the manuscript. SR, NS, SK, and VvK revised the manuscript critically for important intellectual content.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2019.00263/full#supplementary-material

**REFERENCES**

Archer, J. (1973). Tests for emotionality in rats and mice: a review. Anim. Behav. 21, 205–235. doi: 10.1016/0003-3472(73)80065-x

Bandelow, B., and Michaelis, S. M. (2015). Epidemiology of anxiety disorders in the 21st century. Dialog. Clin. Neurosci. 17, 327–335.

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. J. Statist. Softw. 67, 1–48. doi: 10.18637/jss.v067.i01

Bateson, M., and Matheson, S. M. (2007). Performance on a categorisation task suggests that removal of environmental enrichment induces pessimism in captive European starlings (Sturnus vulgaris). Anim. Welf. 16, 33–36.

Beevers, C. G., Gibb, B. E., McGeeary, J. E., and Miller, I. W. (2007). Serotonin transporter genetic variation and biased attention for emotional word stimuli among psychiatric inpatients. J. Abnorm. Psychol. 116, 208–212. doi: 10.1037/0021-843X.116.1.208

Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., and Williams, R. (2009). Vulnerability genes or plasticity genes? Mol. Psychiatr. 14, 746–754. doi: 10.1038/mp.2009.44

Bengel, D., Murphy, D. L., Andrews, A. M., Wichems, C. H., Feltner, D., Heils, A., et al. (1998). Altered brain serotonin homeostasis and locomotorinsensitivity to 3,4-Methylenedioxymethamphetamine (“Ecstasy”) in serotonin transporter-deficient mice. Mol. Pharmacol. 53, 649–655. doi: 10.1124/mol.53.4.649
Brydges, N. M., Leach, M., Nicol, K., Wright, R., and Bateson, M. (2011). The effects of juvenile stress on anxiety, cognitive bias and decision making in adulthood: a rat model. PLoS One 7:e48143. doi: 10.1371/journal.pone.0048143

Brydges, N. M., Leach, M., Nicol, K., Wright, R., and Bateson, M. (2011). Environmental enrichment induces optimistic cognitive bias in rats. Anim. Behav. 81, 169–175. doi: 10.1016/j.anbehav.2010.09.030

Burman, O. H. P., Parker, R. M. A., Paul, E. S., and Mendel, M. T. (2009). Anxiety-induced cognitive bias in non-human animals. Physiol. Behav. 98, 345–350. doi: 10.1016/j.physbeh.2009.06.012

Bussey, T. I., Holmes, A., Lyon, L., Mar, A. C., McAllister, K. A. L., Nithianantharajah, J., et al. (2012). New translational assays for preclinical modelling of cognition in schizophrenia: the touchscreen testing method for mice and rats. Neuropharmacology 62, 1191–1203. doi: 10.1016/j.neuropharm.2011.04.011

Canli, T., and Lesch, K.-P. (2007). Long story short: the serotonin transporter in emotion regulation and social cognition. Nat. Neuroscience 10, 1103–1109. doi: 10.1038/nn1964

Carola, V., Frazzetto, G., Pascucci, T., Audero, E., Puglisi-Allegra, S., Cabib, S., et al. (2008). Identifying molecular substrates in a mouse model of the serotonin transporter x environment risk factor for anxiety and depression. Biol. Psychiatry 63, 840–846. doi: 10.1016/j.biopsych.2007.08.013

Carroll, J. C., Boyce-Rust, J. Y., Millstein, R. Y., Yang, R. J., Lesch, K.-P., Crawley, J. N., and Murphy, D. L., et al. (2007). Effects of mild early life stress on abnormal emotion-related behaviors in 5-HTT knockout mice. Behav. Genet. 37, 214–222. doi: 10.1007/s10519-006-9129-9

Caspi, A., Harriri, A. R., Holmes, A., Uher, R., and Moffitt, T. E. (2010). Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am. J. Psychiatr. 167, 509–527. doi: 10.1176/appi.ajp.2010.09101452

Caspi, A., and Moffitt, T. E. (2006). Gene–environment interactions in psychiatry: joining forces with neuroscience. Nat. Rev. Neurosci. 7, 583–590. doi: 10.1038/nrn1925

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386–389. doi: 10.1126/science.1083968

Crawley, J. N., and Goodwin, F. K. (1980). Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. 13, 167–170. doi: 10.1016/0091-3997(80)90067-2

Destreza, A., Deiss, V., Leterrier, C., Calandreau, L., and Boissy, A. (2014). Repeated punishment in Mesocricetus auratus positive judgement bias for mildly (but not truly) ambiguous cues to reward. Anim. Behav. 81, 169–175. doi: 10.1016/j.anbehav.2010.09.030

Destrez, A., Deiss, V., Leterrier, C., Calandreau, L., and Boissy, A. (2014). Repeated punishment in Mesocricetus auratus positive judgement bias for mildly (but not truly) ambiguous cues to reward. Anim. Behav. 81, 169–175. doi: 10.1016/j.anbehav.2010.09.030

Horner, A. E., Heath, C. J., Ivosofle-Eide, M., Kent, B. A., Kim, C. H., Nilsson, S. R. O., et al. (2013). The touchscreen operant platform for testing learning and memory in rats and mice. Nat. Protoc. 8, 1961–1984. doi: 10.1038/nprot.2013.122

Hurst, J. L., and West, R. S. (2010). Taming anxiety in laboratory mice. Nat. Methods 7, 825–826. doi: 10.1038/nmeth.1500

Jansen, F., Heimig, R. S., Lewejohann, L., Touma, C., Palme, R., Schmitt, A., et al. (2010). Modulation of behavioural profile and stress response by 5-HTT genotype and social experience in adulthood. Behav. Brain Res. 207, 21–29. doi: 10.1016/j.bbr.2009.09.033

Jones, S., Neville, V., Higgs, L., Paul, E. S., Dayan, P., Robinson, E. S. J., et al. (2018). Assessing animal affect. An automated and self-initiated judgement bias task based on natural investigative behaviour. Sci. Rep. 8:116. doi: 10.1038/s41598-018-3057-1

Kästner, N., Richter, S. H., Lesch, K.-P., Schreiber, R. S., Kaiser, S., and Sachser, N. (2015). Benefits of a “vulnerability gene”? A study in serotonin transporter knockout mice. Behav. Brain Res. 283, 116–120. doi: 10.1016/j.bbr.2015.01.031

Kloke, V., Schreiber, R. S., Boddem, C., Möllers, J., Ruhmann, H., Kaiser, S., et al. (2014). Hope for the best or prepare for the worst? Towards a spatial cognitive bias test for mice. PLoS One 9:e105431. doi: 10.1371/journal.pone.0105431

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., Boer, S. F., de Flügge, G., Korte, S. M., et al. (2011). Stress revisited: a critical evaluation of the stress concept. Neurosci. Biobehav. Rev. 35, 1291–1301. doi: 10.1016/j.neubiorev.2011.02.003

Kosten, N., Vuigk, I., Garcia Rodriguez, L., Kästner, N., Kaiser, S., Sachser, N., et al. (2019). Technology or ecology? New tools to assess cognitive judgement bias in mice. Behav. Brain Res. 362, 279–287. doi: 10.1016/j.bbr.2019.01.021

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. J. Statist. Softw. 82, 1–26. doi: 10.18637/jss.v082.i13

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front. Psychol. 4:863. doi: 10.3389/fpsyg.2013.00863

Lenth, R. V. (2016). Least-squares means: the r package lsmeans. J. Statist. Softw. 69, 1–33. doi: 10.18637/jss.v069.i01

Lesch, K.-P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274, 1527–1531. doi: 10.1126/science.274.5921.1527

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92, 180–185.

Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. Pharmacol. Ther. 46, 321–340. doi: 10.1016/0163-7258(90)90021-s

Malinen, A. S., Palme, R., Richetto, J., Muzzillo, C., Richter, S. H., Vogt, M. A., et al. (2016). Daily exposure to a touchscreen-paradigm and associated food restriction evokes an increase in adrenocortical and neural activity in mice. Horne. Behav. 81, 97–105. doi: 10.1016/j.hbeh.2016.03.009

Marashi, V., Barnekow, A., Ossendorf, E., and Sachser, N. (2003). Effects of different forms of environmental enrichment on behavioral, endocrinological,
and immunological parameters in male mice. *Horm. Behav.* 43, 281–292. doi: 10.1016/S0018-506X(03)00002-3

Mathews, A., and MacLeod, C. (1994). Cognitive approaches to emotion and emotional disorders. *Ann. Rev. Psychol.* 45, 25–50. doi: 10.1146/annurev.psych.45.1.25

Mathews, A., and MacLeod, C. (2005). Cognitive vulnerability to emotional disorders. *Ann. Rev. Clin. Psychol.* 1, 167–195. doi: 10.1146/annurev.clnpsy.1.102803.143916

Mazzanti, C. M., Lappalainen, J., Long, J. C., Bengel, D., Naukkarinen, H., Eggert, M., et al. (1998). Role of the serotonin transporter promoter polymorphism in anxiety-related traits. *Arch. Gen. Psychiatry* 55, 936–940. doi: 10.1001/archpsyc.55.10.936

Murphy, D. L., Li, Q., Engel, S., Wichems, C. H., Andrews, A. M., Lesch, K.-P., et al. (2001). Genetic perspectives on the serotonin transporter. *Brain Res. Bull.* 56, 487–494. doi: 10.1016/s0361-9230(01)00622-0

Papciak, J., Popik, P., Fuchs, E., and Rygula, R. (2013). Chronic psychosocial stress makes rats more ‘pessimistic’ in the ambiguous-cue interpretation paradigm. *Behav. Brain Res.* 256, 305–310. doi: 10.1016/j.bbr.2013.08.036

Pellow, S., Chopin, P., File, S. E., and Briley, M. (1985). Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety n the rat. *J. Neurosci. Methods* 14, 149–167. doi: 10.1016/0165-0270(85)90031-7

Plomin, R., Owen, M. J., and McGuffin, P. (1994). The genetic basis of complex human behaviors. *Science* 264, 1733–1739. doi: 10.1126/science.8209254

Plomin, R., Scheier, M. F., Bergman, C. S., Pedersen, N. L., Nesselroade, J. R., and McClearn, G. E. (1992). Optimism, pessimism and mental health: a twin/adoption analysis. *Pers. Individ. Differ.* 13, 921–930. doi: 10.1016/0191-8669(92)90009-E

Rafa, D., Kregiel, J., Popik, P., and Rygula, R. (2016). Effects of optimism on gambling in the rat slot machine task. *Behav. Brain Res.* 300, 97–105. doi: 10.1016/j.bbr.2015.12.013

Ramos, A., and Mormède, P. (1998). Stress and emotionality: a multidimensional and genetic approach. *Neurosci. Biobehav. Rev.* 22, 33–57. doi: 10.1016/S0149-7634(97)00001-8

Richter, S. H., Schick, A., Hoye, C., Lankisch, K., Gass, P., and Vollmayr, B. (2012). A glass full of optimism: enrichment effects on cognitive bias in a rat model of depression. *Cogn. Affect. Behav. Neurosci.* 12, 527–542. doi: 10.3758/s13415-012-0101-2

Richter, S. H., Vogel, A. S., Ueltzhöffer, K., Muzzillo, C., Vogt, M. A., Lankisch, K., et al. (2014). Touchscreen-paradigm for mice reveals cross-species evidence for an antagonistic relationship of cognitive flexibility and stability. *Front. Behav. Neurosci.* 8:154. doi: 10.3389/fnbeh.2014.00154

Roelofs, S., Boleij, H., Nordquist, R. E., and van der Staya, F. J. (2016). Making decisions under ambiguity: judgment bias tasks for assessing emotional state in animals. *Front. Behav. Neurosci.* 10:119. doi: 10.3389/fnbeh.2016.00119

Rygula, R., Golebiowska, J., Kregiel, J., Holuj, M., and Popik, P. (2015). Acute administration of lithium, but not valproate, modulates cognitive judgment bias in rats. *Psychopharmacology* 232, 2149–2156. doi: 10.1007/s00213-014-3847-0

Rygula, R., Papciak, J., and Popik, P. (2013). Trait pessimism predicts vulnerability to stress-induced anhedonia in rats. *Neuropsychopharmacology* 38, 2188–2196. doi: 10.1038/npp.2013.116

Salmeto, A. L., Hymel, K. A., Carpenter, E. C., Brilot, B. O., Bateson, M., and Sufka, K. J. (2011). Cognitive bias in the chick anxiety-depression model. *Brain Res.* 1373, 124–130. doi: 10.1016/j.brainres.2010.12.007

Talpos, J., and Steckler, T. (2013). Touching on translation. *Cell Tissue Res.* 354, 297–308. doi: 10.1007/s00441-013-1694-7

Treat, D., and Fundytus, M. (1989). Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacol. Biochem. Behav.* 31, 959–962. doi: 10.1016/0091-3057(88)90413-3

van den Hove, D., Jakob, S. B., Schrart, K.-G., Kenis, G., Schmitt, A. G., Kneitz, S., et al. (2011). Differential effects of prenatal stress in 5-Htt deficient mice: towards molecular mechanisms of gene × environment interactions. *PLoS One* 6:e22715. doi: 10.1371/journal.pone.0022715

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.