Research report

Social status and day-to-day behaviour of male serotonin transporter knockout mice

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\textbf{ABSTRACT}

Humans differing in the amount of serotonin transporter (5-HTT) are known to be differentially prone to neuropsychiatric disorders. Genetically modified mice eliciting abrogated transporter function display a number of corresponding phenotypic changes in behavioural tests. However, a characterisation of the effects of serotonergic malfunction on the day-to-day life is still missing. Yet, this is precisely what an animal model is needed for in order to be meaningful for translation into human anxiety disorders.

Homozygous 5-HTT knockout mice, heterozygous 5-HTT mice, and wild-type controls were housed in groups of males of the same genotype in spacious and richly structured cages. This enriched environment allowed the animals to show a wide variety of spontaneous behavioural patterns quantified by a trained experimenter. Additionally the mice could emigrate from the cages through a tunnel and a water basin. The results revealed unaltered daily behaviour in heterozygous mice. In knockouts, however, reduced locomotion, increased socio-positive behaviour, and reduced aggressive behaviour were observed. Nevertheless, all groups showed a significant amount of aggressive behaviour and there were no differences regarding the establishment of dominance relationships, emigration, and the number of animals remaining in their groups.

In a second step, pairs of heterozygous and wild-type males and pairs of knockout and wild-type males were brought together in order to assess their ability to obtain a dominant social position in a direct encounter. Heterozygous mice did not differ from wild-type mice but knockout mice were significantly inferior in obtaining the dominant position.

In addition to confirming multiple effects of abolished 5-HTT function in a real life situation, this study supports the central role of the 5-HTT in the control of social interactions.

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1. Introduction

Vulnerability to anxiety disorders is due to multiple genes of modest effect in interaction with each other and in conjunction with environmental events [34]. Thereby, the serotonergic system has emerged to play a key regulatory role on central nervous system processes that are hypothesised to be dysregulated in psychiatric diseases. Coppen already postulated in 1967 that there is a causal association between brain monoamines and affective disturbances and pointed to significantly decreased levels of 5-hydroxyindoles in the cerebrospinal fluid, the main metabolite of serotonin (5-hydroxytryptamin, 5-HT) in the human body [11]. 5-HT is a monoamine neurotransmitter, involved in a large number of physiological processes including mood, cognition, motor system, circadian rhythms, endocrinological functions, food intake, thermoregulation, and perception of pain [46]. Moreover, the signalling molecule is an important regulator of early brain development and adult neuroplasticity, including cell proliferation, migration, differentiation, and synaptogenesis, thereby 5-HT enables functions that regulate emotions throughout life [33].

There are no fewer than 15 structurally and pharmacologically distinct mammalian receptor subtypes for 5-HT [3,32]. In contrast to the diversity of 5-HT receptors the reuptake of 5-HT from the synaptic cleft into the presynaptic neuron is mediated by only one single protein, the 5-HT transporter (5-HTT). Thus, 5-HTT is a key regulator of serotonergic activity by removing 5-HT from the synaptic cleft and thereby terminating the effects of 5-HT on any kind of 5-HT receptor. The human gene coding for 5-HTT is modulated\footnote{Corresponding author at: Department of Behavioural Biology, University of Muenster, Badestraße 13, D-48149 Muenster, Germany. E-mail address: Ljohann@phenotyping.de (L. Lewejohann).}

\footnote{These authors contributed equally to this work.}
by a length variation of a repetitive element, termed the 5-HTT gene-linked polymorphic region (5-HTTLPR). This region is most commonly composed of either 14 (short allele) or 16 (long allele) repeated elements, although alleles with more repeated elements or other variants occur [17,35,49]. The short variant of the polymorphism causes a reduction in the transcriptional efficiency of the 5-HTT promoter, resulting in decreased 5-HTT expression and therefore a reduced 5-HT reuptake from the synaptic cleft into the presynaptic cell compared to the long variant [36,49]. The low-expressing 5-HTTLPR short variant has been implicated in higher scores of neuroticism, depression, and stress reactivity [36]. Furthermore it is associated with a heightened trait-anxiety, dysphoria and an exaggerated neural response to fear [8,24,33]. Carriers of one or two copies of the short allele exhibit more depressive symptoms, diagnosable depression and suicidality in relation to stressful life events than individuals homozygous for the long allele ([10] but see also [55]).

The evidence of a connection between mood disorders and genetic variations of the 5-HTT led to the generation of 5-HTT knockout mice with a targeted inactivation of the 5-HTT function [6]. In contrast to the human 5-HTT gene a 5-HTTLPR-like sequence is absent in mice [18]. Homozygous knockouts (KO) completely lack the 5-HTT, heterozygous mice (HET) show a reduced 5-HTT density of about 50%, while wild-type controls (WT) express normal levels of 5-HTT. Comparable to the phenomena known from humans these mice express varying phenotypes, depending on their genotypes. In brief, the loss of functional 5-HTT results in increased anxiety [19,21,25–27], reduced locomotor [22,27] and reduced home-cage activity [15,58], deficits in extinction recall of conditioned fear memory [61], decreased social investigation [47], and decreased aggressive behaviour [22]. Taken together 5-HTT KO mice are accepted to be a suitable model to robustly measure effects of 5-HTT depletion [28,50].

The behavioural analyses that were carried out with this model so far were done with mice housed in small standard sized cages. Most of the behavioural analyses were conducted in artificial apparatuses and thereby the mice were subjected to a challenging situation. Although such tests are fundamental in the characterisation of animal models, translation into human behaviour would profit from an additional in-depth characterisation of how the differential expression of 5-HTT affects the day-to-day life of these mice in their home environment.

In the first part of the present study groups of male mice of all three genotypes were housed in spacious and richly structured terraria (Fig. 1A) allowing the animals to show a variety of species specific behaviour. Additionally, the mice were given the opportunity to escape from agonistic encounters (Fig. 1B) and thus dispersal could be studied in an animal model of serotonergic dysfunction for the first time. We hypothesised that the stable and well documented phenotypic effects known for 5-HTT KO mice would also lead to noticeable effects on the day-to-day life of these mice in their home environment.

In the second part of the present study groups of male mice of all three genotypes were housed in spacious and richly structured terraria (Fig. 1A) allowing the animals to show a variety of species specific behaviour. Additionally, the mice were given the opportunity to escape from agonistic encounters (Fig. 1B) and thus dispersal could be studied in an animal model of serotonergic dysfunction for the first time. We hypothesised that the stable and well documented phenotypic effects known for 5-HTT KO mice would also lead to noticeable effects on the day-to-day life of the animals, especially regarding activity and aggressive behaviour [50]. Concerning HET 5-HTT mice, we did not expect a conspicuous phenotype, however, there are indications for a gene-dosage effect, which might lead to an intermediate phenotype between KO and WT [27].

In the second part of the investigation, males from different terraria were subjected to dominance encounters in a competitive dyadic housing situation. The pairings consisted of either a WT and a KO or of a HET and a WT. Since the dominance ranks of all individual mice were known from the characterisation of their
day-to-day behaviour in the terraria, pairs could be matched for social status. Therefore all mice had equal opportunities to win the encounter with regard to their previous experience, but they differed regarding their genotype. We expected that the outcome of this test would be influenced especially by anxiety-related and aggressive behaviour rendering WT mice the odds-on favourite.

2. Materials and methods

2.1. Animals

A total of 20 knockout, 20 heterozygous, and 40 wild-type male mice of the genetically modified 5-HTT line were used. The study was conducted in two replicates using identical experimental designs but different genotypes. In the first run, WT were compared with HET and in the second run WT were compared with KO. All animals originated from the internal stock of 5-HTT mice bred at the Department of Behavioural Biology at the University of Muenster, Germany. The original breeding stock was obtained from the Department of Psychiatry at the University of Wuerzburg, Germany, where the 5-HTT KO mice were generated and backcrossed for >10 generations on a C57BL/6 genetic background [6]. The animals included in this study derived from 39 different litters resulting from matings between heterozygous 5-HTT mice. Pups were weaned on day 21 and separated by gender. Genotyping was accomplished using tissue samples to extract genomic DNA amplified by PCR. Subsequently genotypes were identified by gel electrophoresis of DNA-fragments of either 225 bp (WT), 272 bp (KO) or both (HET).

Testing animals were maintained in sibling groups until they were transferred to an enriched housing and subjected to the investigation of their spontaneous behaviour at an age of 66±15 days. The housing room was maintained at a 12 h light/dark cycle (lights on at 08:00 h) at a temperature of 22±3°C.

2.2. Housing and emigration

For the observation of the spontaneous behaviour four groups consisting of five 5-HTT KO mice each, four groups of five HET 5-HTT mice, and eight groups of five WT mice (four groups per batch) were set up. The mice were colour marked on the tail and on both ears and placed into spacious and richly structured glass terraria (Allspan, Höveler GmbH & Co. KG, Langenfeld, Germany). In each terrarium an upper floor realised by a plastic plain (45 cm × 33 cm) installed 25 cm above the ground that was accessible by stairways on two sides. One of the stairways led to the floor, the other one led to the metal lid of the enriched cage from where a third stairway led to the ground. Additionally, a wooden climbing tree of 34 cm height and paper towels as nesting material were placed in each terrarium. Food (Altromin 1324, Altromin GmbH, Lage, Germany) and water were available ad libitum at the inner cage as well as at the second floor. Oat flakes were fed occasionally as nutritional enrichment. Mice could emigrate from the terraria by passing through a Plexiglas tube, swimming through a water basin, and finally entering an emigration cage also providing food and water [Fig. 1B] [13,31,37]. This setup provided the possibility to leave the glass terrarium and thus to withdraw from social pressure, e.g., in situations of agonistic interactions with a dominant conspecific. Whenever an emigration event occurred, the animal was allowed to stay in the emigration cage for 24 h and then was placed back into the terrarium. If the same mouse emigrated three times in a row, it was housed individually until the beginning of the second part of the experiment. To preserve the experience of being subordinate, the emigrant was placed back in the terrarium once a week and subsequently returned to standard housing after the first emigration.

2.3. Dominance structure

When two mice met in agonistic interactions (see Table 1), the winner and loser were noted. Subsequently these data were used to calculate the dominance rank of each individual in a terrarium using Elo-rating [1,37]. In brief, this method estimates the rank of individuals over periods of time by taking into account different propensities to win or lose on the basis of the strength of an individual, known from the outcomes of previous interactions. A major advantage of this technique is that it thereby allows for the rapid monitoring of changes in the dominance structure of a group.

2.4. Day-to-day behaviour

Before observational sessions started, the mice were allowed to habituate for about one week to the new environment and to their new cage mates. Observations of the spontaneous behaviour of mice were conducted during the activity phases of the light period between 08:00 and 12:00 h in the morning and between 16:00 and 20:00 h in the evening. Each individual was observed for an integrated total of at least 90 min in bouts of 1–5 min observed within three weeks. The time schedule of the observations was determined by the activity of the mice. Whenever more than one mouse was active, the individual with the least time recorded so far was chosen. Two sessions observing the same individual were never conducted with less than 15 min interval. In case of a mouse being invisible or inactive for more than 30 s, the observation session was ended for that individual and the time without the 30 s inactivity/invalidity was noted. Approximately two-thirds of the observation time was allotted to the morning sessions when activity was shown to a greater extent. Frequencies of 33 behavioural patterns from various behavioural domains (i.e., agonistic behaviour, socio-positive behaviour, locomotion, and maintenance) were assessed by focal animal sampling and continuous recording [44]. Definitions of behavioural patterns were in accordance with those of former studies [14,39,39] and are listed in Table 1. The genotype of the observed individuals was unknown to the observer during data acquisition.

Table 1

<table>
<thead>
<tr>
<th>Domain</th>
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<tbody>
<tr>
<td>Offensive aggression</td>
<td>Attack</td>
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<td>Crawl over/under</td>
<td>A mouse moves over or beneath the body of a conspecific and stays in this position or crosses the other mouse completely</td>
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<td>A mouse moves the tip of its twitching snout to the anogenital region of a conspecific</td>
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<td>A mouse raises itself on its hindpaws and stretches its snout into the air</td>
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<td>Dig</td>
<td>A mouse moves substrate by a series of fast alternating forepaw movements with its snout lowered into the substrate</td>
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<td>Locomotor activities</td>
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2.5. Competitive dyadic housing

After recording of spontaneous behaviour and determination of rank positions within groups of the same genotype, the mice were subjected to dyadic encounters. Therefore two mice of the same status (dominant, subordinate, emigrant) but of different genotypes were housed together in a cage (38 cm × 21 cm × 15 cm) connected to an emigration cage (22 cm × 16 cm × 14 cm) via plastic tubes and a water basin (Fig. 1C). The number of pairings was limited due to the constraint of using only individuals with equivalent social status but different genotypes. Accordingly, 16 WT were paired with HET in the first batch and 11 WT were paired with KO in the second batch. All individuals were weighted before being subjected to the encounter and weight differences were kept as low as possible with a mean weight difference of 2.1 ± 1.4 g (SD). Whenever an emigration event occurred, the emigrant was allowed to stay in the emigration cage for 24 h and then was placed back into the cage where the dominant male resided. This procedure was repeated until a mouse emigrated three times in a row indicating its subordinate position. When no emigration event occurred for four days, a female was introduced to both males for 24 h in order to increase competition. If still no animal emigrated, the encounter was scored as a draw. If one of the opponents exhibited severe injuries without emigrating, the encounter was stopped and the injured male was scored as the subordinate one.

The presented work complies with current regulations covering animal experimentation in Germany. The experiments were approved by the competent local authority as well as by the ‘Animal Welfare Officer’ of the University of Muenster.

2.6. Statistical analysis

Graphs were created and statistics calculated using the R software package [54]. Data from observations of spontaneous behaviour were analysed using non-parametric statistics [56] due to the ordinal scaling of behavioural data. Differences between groups were analysed using unpaired Wilcoxon exact rank sum tests. Proportions of animals emigrating, remaining, or being removed to prevent severe injury were compared using Fisher’s exact test for count data. Survival analysis was carried out using log-rank statistics and the proportions of males grouped by genotypes winning or losing in dyadic encounters were analysed using Exact Binomial tests. For all data, a significance level of α = 0.05 was chosen.

3. Results

3.1. Continuance, emigration, and survival

A total of 16 terraria comprising five male mice each were included in the series of observations. Of the first batch of 40 animals comparing initially four groups of five WT with four groups of five HET a total of 28 animals remained in the terraria (Fig. 2). In the WT group, six animals emigrated. In the HET group, four males emigrated, one died and one mouse was removed from its group due to preventing severe injury. Thus data from 14 WT and 14 HET were included into the analysis of spontaneous behaviour. In the second batch of 40 animals initially comparing four groups of five WT with four groups of five KO only 23 animals remained in the terraria until the end of the observations (Fig. 2). Two WT animals died during the investigation and two WT and seven KO mice were severely bitten and excluded to prevent further injury. One KO mouse and two WT mice left their terrarium by repeated emigration. As observation was only conducted if at least two animals stayed in a terrarium one WT mouse and two KO mice that each occupied a terrarium on their own had to be excluded. Finally, data of 13 WT and 10 KO mice were recorded and used for further analysis of the spontaneous behaviour. Fisher tests on proportions of animals staying in the terraria revealed no significant differences between the four groups (p = 0.93). The same was true for the proportions of animals leaving the terraria by emigration (p = 0.173). The number of mice that had to be taken out to prevent them from severe injury, however, differed significantly between the groups (p = 0.008) most likely due to the high count of KO in this category. The estimation of the survival ratio (including emigration and males that were taken out due to severe bite marks) revealed neither statistical difference between WT and HET (Exact log-rank test, Z = 0.2, p = 0.84) nor between WT and KO (Exact log-rank test, Z = −0.52, p = 0.61).

3.2. Dominance structure

When two mice met in agonistic interactions, the winner and loser were noted and data were analysed using ELO-rating to describe the course of the dominance relationship. The total number of agonistic interactions counted was 1028 with a mean of 73 agonistic interactions per terrarium. In each terrarium a dominant male could unequivocally be determined by this technique. A sample ELO-rating of a group of five KO mice is shown in Fig. 3. ELO-points of dominant males ranged from 1342 to 1643 with a mean of 1468. Losers had an average of 843 ELO-points ranging from 556 to 1151. All 13 males that left the terraria by emigration were subdominant, the same was true for the nine males that had to be taken out to prevent them from severe injury and for the three males that
were found dead. Thus, being subdominant came obviously with a particular risk.

With regard to the social structure and number of interactions there were no obvious differences between the genotypes. Pooled data of all genotypes revealed that, apart from self-evident differences in agonistic behaviour, dominant males showed significant more exploratory behaviour (Exact Wilcoxon test, \(N_{\text{dom}} = 13, N_{\text{sub}} = 36, W = 388, p = 0.0003\)) more social exploration (Exact Wilcoxon test, \(N_{\text{dom}} = 13, N_{\text{sub}} = 36, W = 323, p = 0.044\)) and a trend for general increased locomotor activities (Exact Wilcoxon test, \(N_{\text{dom}} = 13, N_{\text{sub}} = 36, W = 308, p = 0.096\)).

3.3. Day-to-day behaviour

Since the study was conducted in two separate batches, data of two distinct cohorts of WT mice were obtained. Although procedural details were strictly standardised between the two batches differences between the two WT groups occasionally occurred (Fig. 4). For this reason HET and KO were compared to the pre-assigned WT group of the respective batch but no comparison between HET and KO was calculated. Comparing HET with WT mice revealed only marginal differences that did not reach statistical significance (Figs. 4–8). In contrast, KO mice differed significantly from WT in many behavioural domains. KO mice showed significantly more socio-positive behavioural patterns than WT (Exact Wilcoxon test, \(W = 114.5, p = 0.001\); Fig. 4) and more self-grooming (Exact Wilcoxon test, \(W = 113, p = 0.002\); Fig. 5). Offensive aggressive behavioural patterns on the other hand were significantly reduced in KO mice compared with WT (Exact Wilcoxon test, \(W = 32, p = 0.036\); Fig. 6). Regarding locomotor activity, KO mice showed a significant reduction (Exact Wilcoxon test, \(W = 17, p = 0.002\); Fig. 7). Yet, social exploration did not differ between the WT and KO (Exact Wilcoxon test, \(W = 54, p = 0.52\); Fig. 8).

3.4. Competitive dyadic housing

In the first batch pairing WT with HET mice, four pairings of dominant animals, seven pairings of subdominant animals and five pairings of emigrants were arranged. In the second batch four pairings of dominant animals and seven pairings of subdominant animals were arranged between WT and KO mice. All animals were weighed before being paired to keep differences as low as possible.
Fig. 7. Locomotor activities. Behavioural patterns indicative for locomotion were measured by direct observation of each individual mouse living in groups of two to five animals in spacious terraria. Each box represents the 25th–75th percentile, and the horizontal line across the box is the median (50th percentile). Whisker lines extending below and above represent the extremes lying within 1.5 times the interquartile range (box height). 5-HTT knockout mice (KO) differed significantly from wild-types (WT). Heterozygous 5-HTT mice (HET) did not differ from wild-types and there was no significant difference between the two batches of WT. Statistics: NKO :10, NWT :13, NHET :14, unpaired Wilcoxon test (two-tailed); \*\*p < 0.01.

Overall, there was no significant difference between the weights of males from different groups.

WT mice won significantly more dyadic encounters than KO mice (Exact Binomial test, p = 0.022; Fig. 9) but HET mice did not differ significantly from WT mice regarding their propensity to win an encounter (Exact Binomial test, p = 1; Fig. 9).

4. Discussion

The serotonin transporter plays a key regulatory role on central nervous system processes that seem to be dysregulated in neuropsychiatric disorders like anxiety and depression [8,48]. While in rhesus macaques the short allele of the 5HTTLPR is used as a natural occurring model to resemble the reduced 5-HTT in humans, knockout mice and knockout rats were generated to systematically study the reduction or complete absence of the 5-HTT [4,5,28]. So far, more than 50 different phenotypic changes have been related to the deletion of the 5-HTT [48]. Noteworthy, in a first approach of direct observation of the behaviour in the home-cages no significant differences were found between the three genotypes [47]. In that study, the mice were housed in smaller cages and a limited observation time of 60 min per group included also periods the animals were not active. In our approach, the mice were given more space and observation time was increased to 90 min of focal animal sampling of mice that were active.

4.1. Dominance structure and emigration

5-HTT is not a prerequisite to build up a social hierarchy but may make it easier to cope with unfavourable conditions. In a spacious home-environment, there were no obvious differences between the genotypes concerning the dominance structure. 5-HTT mice of all three genotypes established a common type of social hierarchy as it has been described for this species in many studies [37,41,52,53,59]. Hence, the reduction or the complete lack of the 5-HTT does not impair the ability to form a social hierarchy. Surprisingly, despite obvious inferiority almost none of the subordinate 5-HTT KO males emigrated from the terraria. Indeed, seven subordinate males had to be removed from two of the terraria in order to prevent severe injury. The refusal to emigrate from unfavourable social conditions is in line with the overall increased anxiety-like behaviour and observations of increased depressive-like immobility.
At the dawn of a new day, when the animals were moved from the cages to the terraria, the dominant 5-HTT KO males took control in all terraria, regardless of the genotype of the subordinate animals. The complete lack of 5-HTT led to altered day-to-day behaviour whereas the daily behaviour of HET mice was largely unaffected. We conducted the study in two batches, each of them containing a separate group of WT mice as controls. Strikingly, WT mice from the different batches differed significantly regarding behavioural patterns summarised as socio positive behaviour. It is known from previous studies that behavioural data may vary between different laboratories and observers [38] and therefore a variance between different batches did not hit us completely by surprise. To avoid observer-reliability issues [16] we chose to have only one trained observer conducting all direct observations, although we admit that it would have been advantageous to include all three genotypes in the same batch. Nevertheless, for these reasons we did not include a direct comparison of 5-HTT KO to 5-HTT HET mice regarding their day-to-day behaviour.

In the first batch, comparing HET with WT, the results point to a widely unaltered behavioural phenotype when mice were housed in groups in spacious terraria. HET mice did not exhibit any alterations that suggest major impairments by reduced 5-HTT function, maybe due to compensatory mechanisms that ameliorate sensory functions [22,27,62]. It is therefore suggested, that the behavioural phenotype of 5-HTT KO mice is influenced by hypolocomotion in combination with anxiety due to pleiotropic effects of targeted 5-HTT disruption [24,29].

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### 4.2. Locomotion

5-HTT KO mice were hypoactive compared to WT mice. The dramatic reduction of locomotor activity in 5-HTT KO mice compared with WT mice is in overall agreement with former studies [50]. For example significantly lower levels of home-cage activity, an inhibited exploratory locomotion in tests like the elevated plus maze, the open field or the light-dark exploration test were observed in 5-HTT KO mice compared with HET and WT mice [23]. Motor coordination and sensorimotor integration in itself, however, appear unaltered in KO mice as well as gross physical, neurological and sensory functions [22,27,62]. It is therefore suggested, that the behavioural phenotype of 5-HTT KO mice is influenced by hypolocomotion in combination with anxiety due to pleiotropic effects of targeted 5-HTT disruption [24,29].

### 4.2.2. Self-grooming

5-HTT KO mice displayed higher frequencies of self-grooming than WT mice. This contradicts results from an earlier study with no differences between WT and KO [27]. Grooming in general is a complex and essential ritual in the rodent behavioural repertoire related to comfort [57]. The higher frequencies of self-grooming may correspond to the reduced aggressiveness observed in the cages of 5-HTT KO mice. Thus, in the two terraria in which a stable dominance hierarchy was established and no severe injuries were observed, the 5-HTT KO mice were most likely under less constraint.

### 4.2.3. Socio-positive behaviour and social exploration

5-HTT KO mice were not less social than WT mice. It was proposed earlier that serotonin transporter knockout mice might be a suitable model for autism spectrum disorders due to indications of reduced social behaviour in social preference tasks [27,28,47]. Notably, all these results were obtained in paradigms in which the mice were confronted with unfamiliar conspecifics in an unknown environment. In our observations, 5-HTT KO mice expressed overall more socio-positive behaviours than WT controls, when social behaviour was assessed in stable groups with well-established dominance hierarchies. Maybe tests on sociability are more likely to interfere with anxiety-related behaviour when they are conducted in a novel environment.

### 4.2.4. Aggression

5-HTT KO mice displayed less aggressive behaviour than WT mice in established social groups. Individual levels of aggression are based upon the interaction of genetic and environmental factors [45,60]. Neurotransmitters rank among the main modulators and serotonin has been implicated in the neural control of aggressive behaviour in humans, nonhuman primates, as well as in rodents [7,12,20]. Pharmacological manipulations of the extracellular level of 5-HT reduce aggressive behaviour in several species [51]. Considering the four-fold to sixfold increase in basal levels of extracellular 5-HT in 5-HTT KO mice, attention was also attracted to their aggressive behaviour. Using the resident-intruder paradigm, 5-HTT KO mice indeed are characterised by a lower aggressiveness. Furthermore 5-HTT KO mice are insensitive to repeated exposure to the intruder, while this procedure results in an increase of aggression in HET 5-HTT and WT mice [23]. The results of reduced aggression obtained in our study are thus in line with previous studies [12,22,24]. Nevertheless, 5-HTT KO mice were equally able to establish and maintain dominance relationships including significant amounts of aggressive behaviour. What is more, in two of the terraria the dominant males severely injured their conspecifics. Thus, we highlight that the loss of the 5-HTT did not necessarily bring about a peaceful behavioural profile in groups of males of the same genotype.

### 4.3. Competitive dyadic housing

5-HTT KO mice were inferior to WT mice in a direct confrontation. In dyadic housing with WT mice, HET 5-HTT mice were not at all affected in their propensity to obtain a dominant position. In contrast, the complete lack of 5-HTT significantly reduced the chance of 5-HTT KO mice winning over WT mice. Consequently, in only one out of 11 encounters a 5-HTT KO male became dominant over a WT mouse. Consistent results were previously obtained in a resident-intruder paradigm with 5-HTT KO showing a reduced latency to attack the opponent and their aggression did not even increase after repeated exposure to an intruder [22]. In a direct confrontation with an unknown male the reduced aggressiveness seems to be a severe disadvantage for the 5-HTT KO males. Additionally, a state of behavioural despair that has been observed in 5-HTT KO mice [2,61] might dispose them to endure such unpleasant situations rather passively. We controlled for weight and equivalent social status obtained prior to the encounter, in order to prevent individuals from taking advantage from its pre-conditions. Nevertheless, WT males might have been involved in more agonistic interactions during their stay in the terraria, resulting in an advantage of training history. Therefore, a direct competition in mixed genotype groups might finally clarify whether or not and under what kind of conditions a certain genotype is advantageous.
Interestingly, it was found that in macaques an impaired serotonin signalling is indeed associated with lower rank in a social group [4]. However, in humans the 5-HTTLPR genotype does not correlate with the socio-economic status per se. Nonetheless, individuals with higher socio-economic status and at least one short 5-HTTLPR allele elicit higher serotonin responsivity indicating an interaction of 5-HTTLPR genotype with socio-economic status [42].

5. Conclusions

5-HTT KO mice display a number of changes in their spontaneous behaviour in comparison to WT mice. This is in overall accordance to the literature about the 5-HTT KO mouse model. While so far behaviour was mainly observed under challenging testing conditions and therefore rather represents the response to artificial situations, the present study demonstrated considerable changes of the day-to-day behaviour of 5-HTT KO mice for the first time. In a nutshell, many of the phenotypic alterations seen in 5-HTT KO mice seem to derive from the interaction of the anxiety-like and hypolocomotive phenotype of the mouse model. This confirms the key regulatory role of the 5-HTT and the far-reaching consequences if its function is abrogated. In groups of the same genotype, 5-HTT KO mice were equally able to establish and maintain a dominant social position by offensive aggressive behaviour. Once 5-HTT KO mice lived in such an established and thus predictable social environment, they showed less offensive aggressive behaviour and more socio-positive behaviour compared with WT mice in a similar situation. In a direct confrontation between WT mice and 5-HTT KO mice, however, KO mice were significantly inferior to WT mice. Taken together, our results thus emphasise the central role of the serotonergic system in the control of social behaviour.

Disclosure/conflict-of-interest statement

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.

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