Behavioural Brain Research 219 (2011) 291–301

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Away game or home match: The influence of venue and serotonin transporter genotype on the display of offensive aggression

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ARTICLE INFO

Article history:
Received 9 December 2010
Received in revised form 11 January 2011
Accepted 16 January 2011
Available online 22 January 2011

Keywords:
5-HTT
Genotype
Anxiety
Gene × environment interaction
Risk profile
Cost/benefit ratio

ABSTRACT

Aggression can be modulated by both genetic and environmental factors. Here, we analyse how the serotonin transporter (5-HTT) genotype and the environmental situation in which a contest takes place shape the display of offensive aggression. Therefore, male wildtype, heterozygous, and homozygous 5-HTT knockout mice, which are known to differ in inborn levels of anxiety, were confronted three times with a docile opponent in one of three environmental situations: own territory, opponent's territory or neutral area. The main findings were: The frequency of *approaching* the contestant in order to gather information about him depended significantly on the venue but not on the genotype with lowest frequencies in the opponent's territory. The *decision how quickly to attack* the opponent was significantly influenced by the 5-HTT genotype but not by the venue: Homozygous 5-HTT knockout mice showed longest latencies. The *sum of offensive aggression* was significantly influenced by the 5-HTT genotype, the environmental situation, and a genotype by environment interaction. It is likely that, due to their varying genetic predisposition for anxiety, mice of the three genotypes were differentially affected by the aversiveness of the respective venue and the opponent's behaviour, which influenced their decision to display offensive aggression. As a consequence, the amount of aggression shown by homozygous 5-HTT knockout mice was influenced by the venue and the opponent's behaviour, whereas heterozygotes reacted only to the venue. Strikingly, wildtypes behaved always the same way, irrespective of venue and opponent.

1. Introduction

Both genetic and environmental factors are involved in the display of aggressive behaviour [2]. On the molecular level, serotonin (5-HT) signalling turns out to be the major modulator of emotional behaviour including aggression and impulsivity in humans, nonhuman primates, and other mammals [88]. However, it is not sufficiently clarified whether 5-HT generally dampens aggression or if it plays an opposite role in adaptive and escalated forms of aggressive behaviour [17,45,58,66]. Especially the 5-HT transporter (5-HTT), one of the functional components of the 5-HT pathway, has been linked to aggression [22,28,46].

The 5-HTT is a key regulator in serotonergic neurotransmission, removing serotonin from the synaptic cleft into the presynaptic terminal and thus determining the magnitude and duration of postsynaptic receptor-mediated signalling [12,47]. In humans, a repeat length polymorphism in the transcriptional control region of the 5-HTT gene (*SLC6A4*) was found, resulting in allelic variation of 5-HTT expression and function as well as anxiety and depression, but also aggressiveness [5,44,46]. The generation of mice with a targeted disruption of the 5-HTT gene allows to investigate the consequences of its diminished or absent function. The loss of functional changes results in more than 50 different phenotypic changes such as increased anxiety [29,32,36,39] and reduced aggressive behaviour ([31,49]; for a review see [62]).

Some of these alterations may be shaped by environmental influences like early life adversity or negative experiences during adulthood [6,14,29,36]. Up to now, gene by environment interaction studies in 5-HTT knockout mice mainly focused on anxiety and depression-related behaviours, while the study on aggressive behaviour in these mice has largely been disregarded. Neverthe-

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doi:10.1016/j.bbr.2011.01.029

less, this should be of special interest, because several disorders, including depression, personality disorders or drug abuse, that are associated with 5-HTT gene variants in humans display also some form of inappropriate aggression as one of their possible manifestations [35]. Additionally, offensive aggression in rodents seems to be systematically related to angry aggression in people [8].

One important environmental modulator of aggressive behaviour is the place where an agonistic interaction happens [2,40]. Residency status is a well-documented contextual determinant of a fight's outcome [23]. In an individual's own territory, agonistic interactions are in general more escalated [1,77], and in many species, including humans, the probability to win a dispute is higher within the own territory or home range [19,42,59,68,87], a phenomenon called 'home advantage' [15] or 'residence effect' [41].

The aim of the present study was to investigate how 5-HTT genotype and the environmental situation in which a contest takes place shape the display of offensive aggression. Therefore, we investigated the aggressive behaviour of male wildtype, heterozygous, and homozygous 5-HTT knockout mice towards a docile opponent (C3H male) in three different environmental situations: the own territory, which was the home cage of the 5-HTT male, the opponent's territory, which was the home cage of the C3H male and a neutral area, which was a cage unfamiliar to both contestants.

Based on the findings of Holmes et al. [31] and Lewejohann et al. [49], we firstly expected genotype-dependent differences in offensive aggression with lowest levels in homozygous 5-HTT knockout mice (hypothesis 1). We further hypothesised that the environmental situation would have an influence on offensive aggression with higher levels in the own territory of the focal animals (hypothesis 2). Since it is known that the hypothalamic–pituitary–adrenocortical (HPA) and hypothalamic–pituitary–gonadal (HPG) axes as well as the sympathetic-adrenomedullary system (SAS) can be involved in the modulation of aggressive behaviour [9,56,66,75,81], we expected corticosterone and testosterone concentrations (hyptheses 3 and 4) as well as tyrosine hydroxylase activity (hypothesis 5) to differ between the genotypes and environmental situations.

2. Methods

2.1. Animals and housing conditions

5-HTT knockout mice [7] backcrossed into a C57BL/6J genetic background for >10 generations were derived from our local stock. Breeding pairs consisted of heterozygous 5-HTT knockout mice, resulting in wildtype, heterozygous, and homozygous knockout offspring. To distinguish between the genotypes, genomic DNA was extracted from ear tissue. PCR amplicons of 225 bp (wildtypes), 272 bp (homozygous 5-HTT knockout mice) or both (heterozygotes) were identified by agarose gel electrophoresis.

In total, 114 male mice (36 wildtypes, 38 heterozygous 5-HTT knockout mice, and 40 homozygous 5-HTT knockout mice) were used for the behavioural investigations (deviations from these sample sizes were due to technical reasons). After weaning at day 21 ± 1 of age, the animals were housed in groups of two to five littermates and in some rare cases together with same-aged males from other litters in standard cages (Macrolon cages type III, 38 cm × 22 cm × 15 cm). From day 60 ± 2 of age, i.e. 20 ± 2 days before beginning of behavioural testing, all focal animals were housed singly in order to exclude the possible influence of social interactions with conspecifics on offensive aggressive behaviour. This was important since homozygous 5-HTT knockout mice are inferior to wildtype mice in direct confrontations [49]. Housing the mice in groups of littermates with mixed genotypes therefore could have resulted in subdominance of homozygous 5-HTT knockout mice what might have influenced their offensive aggression in the testing procedure.

18 males of the strain C3H (obtained from Harlan Winkelmann GmbH (Borchen, Germany) at an age of 60 days) served as opponents for the assessment of social interest, offensive aggressive,and defensive behaviour (see below). At the time of experiments, the C3H mice were at least 75 days of age. Since C3H males show a very low rate of intermale aggression [37], it was possible to house them in groups of three individuals. These mice were used as opponents in confrontations with wildtype and 5-HTT knockout mice in their own territory and a neutral area. In cases where C3H males served as residents in their own home cage, they were housed singly. Since social isolation is known to increase levels of aggression [57], we thus meant to increase the probability that C3H mice defended their own territory. All experimental animals as well as the opponents were housed in standard polycarbonate cages type III with sawdust as bedding material (Allspan Höveler GmbH & Co. KG, Langenfeld, Germany) and a paper towel. To guarantee that the wildtype, heterozygous, and homozygous 5-HTT knockout mice as well as the single housed C3H mice recognized their home cages as own territories, the cages were not cleaned for one week prior to testing. The housing room was maintained at a 12-h light/dark cycle (lights on at 8.00 a.m.) and at a temperature of $22 \pm 3\,^{\circ}C$. Commercial mouse diet (Altromin 1324, Altromin GmbH, Lage, Germany) and water were available *ad libitum*. Under the housing conditions of our laboratory, mice of all three 5-HTT genotypes regularly interact during the light phase, including aggressive encounters [49]. Therefore, tests were conducted between 8.00 a.m. and 10.00 a.m.

The presented work complies with current regulations covering animal experimentation in Germany and the EU (European Communities Council Directive 86/609/EEC). All experiments were announced to the local authority and were approved by the 'Animal Welfare Officer' of the University of Muenster (reference number: 8.87-50.10.46.08.151).

2.2. Health check

At day 40, a general health check using a standard protocol (see [20,48,72]) was performed to guarantee that only healthy animals were included into the study and that data were not the result of deteriorated physical conditions of the animals. All animals passed the health check, indicating that no differences concerning general health state, sensory functions, reflexes, and motor abilities existed between wildtype, heterozygous, and homozygous 5-HTT knockout mice.

2.3. Experimental design

Social interest as well as offensive aggressive and defensive behaviour was assessed in three different environmental situations. To this purpose, focal animals of all three genotypes were confronted with an unknown (docile) C3H male for three times (days 80 ± 2, 84 ± 2, and 88 ± 2) either in their home cages (own territory), in a neutral cage (neutral area; unfamiliar to both contestants) or in the home cage of the C3H male (opponent's territory). Tests were conducted between 8 a.m. and 10 a.m.

To investigate stress reactivity of wildtype as well as heterozygous and homozygous 5-HTT knockout mice non-invasively, faecal samples were collected before the first and after the second confrontation for investigation of corticosterone metabolites (CM) (see Section 2.5.1).

To determine plasma corticosterone and testosterone levels 5-HTT mice were decapitated immediately after the last confrontation on day 88 ± 2 and trunk blood was collected for further analysis (see Section 2.5.2). Additionally, the adrenal glands (see Section 2.5.3) and the brain were dissected for further investigations.

2.4. Behavioural investigations

In order to assess the agonistic behaviour of the focal animals (5-HTT knockout and wildtype mice) in their own territory, mice stayed in their home cage as residents and a C3H male was introduced into the cage as an intruder on three defined days. Accordingly, to test the agonistic behaviour in the opponent's territory, wildtype as well as heterozygous and homozygous 5-HTT knockout mice were placed as intruders in the home cage of a single housed C3H male. In both test situations the paper towel was removed before starting the behavioural investigations. To generate a neutral area, both contestants were placed simultaneously into an unfamiliar Macrolon cage type III containing sawdust, but no paper towel. Via a video camera and an attached monitor, F.J. observed the confrontation and stopped it immediately when fighting was too escalated to prevent the mice from injury. That means, during the first two confrontations the test lasted 10 min at most, but in some cases it had to be stopped earlier. During the last confrontation, the two contestants were separated after 5 min by a grid that was placed into the middle of the cage and stayed in this cage the remaining 5 min. Thereby, it was guaranteed that blood as well as tissue samples were collected at the same time point (10 min after the beginning of the confrontation) for all individuals. Since not all animals attacked the opponent within the 5 min timeframe, an accurate measurement of the *latency to attack* was not possible in this last confrontation and was thus not determined.

To assess social interest, offensive aggressive and defensive behaviour, a total number of eight behavioural patterns (for definition of behavioural patterns, see Table 1) was recorded for the focal animals of all three genotypes by an experienced observer (F.J.) who remained blind to genotype, using the software Observer XT 8.0 (Noldus Information Technology BV, Wageningen, NL). In addition, three behavioural patterns were recorded (see Table 1) to characterise the aggressiveness of C3H opponents.

Focal animals and C3H mice could be easily distinguished by the different coat colour. Data was collected using *focal animal sampling* and *continuous recording*. To avoid a habituation effect of wildtype, heterozygous, and homozygous 5-HTT knockout mice to one specific C3H opponent, C3H mice were used pseudo-randomly.

Table 1
Description of behavioural patterns.

Behaviour	Definition
Social interest behaviour	
Approaching	Direct movement towards another mouse at a walking or running pace until the distance between both mice is at most one body length.
Offensive aggressive behaviour	
Following	A mouse runs after another mouse, while the head of the following mouse is directed to the backside of the other individual. The maximum distance between the animals is one body length. After stopping in forward motion for at least three seconds the behaviour starts again.
Chasing	*Following* subsequent to an agonistic interaction (attack, bite attack or escalated fight).
Attack[a]	A mouse contacts the body of another mouse with its mouth, making that mouse react with winced movement of either single extremities, the tail or the whole body. Attacks are single countable events of low intensity. (*Latency to attack*: Time that elapses until an attack is performed for the first time by the focal animal. If no attack occurred, the latency was set to the maximal testing time of 10 min.)
Bite Attack[a]	A series of attacks with rushing and leaping at another mouse. As the behaviour is of higher intensity than an attack itself, single attacks are not countable anymore.
Escalated fighting[a]	Physical struggle between two mice which is initiated by an attack and usually involves further attacks, kicking, wrestling, and rolling over and over. In-between, mice locked jaws. (A score was given for each escalated fight from the onset until the mice broke apart.)
Defensive behaviour	
Defensive upright posture	Rearing up on the hind paws and keeping still, with the head up in the air, and the forepaws rigidly stretched out towards another mouse.
Defensive sidewise posture	Rearing up on the hind paws and keeping still, with shoulder and flank presented to another mouse.

Note: For description of behavioural patterns see also [36,53,54]. The latency to attack is given as duration in seconds. For all other behavioural patterns frequencies were recorded. For data analysis frequencies of Following, Chasing, Attack, Bite Attack and Escalated fighting were added to the *sum of offensive aggressive behaviour*.

[a] These behavioural patterns were also assessed for the C3H opponents.

2.5. Endocrinological investigations

2.5.1. Corticosterone metabolites (CM)

2.5.1.1. Sample collection. On days 79 ± 2 and 84 ± 2 (i.e. before the first confrontation and after the second one), faecal samples voided between 4 p.m. and 8 p.m (that is 8–12 h after the confrontation) were collected by placing mice in a standard Macrolon cage with fresh bedding. The time frame was chosen according to Touma et al. [84], who showed that a peak of corticosterone metabolites can be found in faeces 8–12 h after the exposure to a stressor. All faeces were frozen at −20 °C until assayed for corticosterone metabolites (corticosterone is the major glucocorticoid in mice [82]). This procedure resembled the routinely performed transfer to clean cages for animal maintenance and thus was not considered to be associated with stress possibly corrupting subsequent samples.

2.5.1.2. Hormone analysis. The collected faecal samples were analysed for immunoreactive corticosterone metabolites (CM) using an established 5α-pregnane-3β,11β,21-triol-20-one enzyme-immunoassay (EIA). Details regarding development, biochemical characteristics, and physiological validation of this assay are described in [84,85]. Before EIA analysis, the faecal samples were homogenised and aliquots of 0.05 g were extracted with 1 ml of 80% methanol. The intra- and inter-assay coefficients of variation were 9.1% and 14.3%, respectively. Samples of less than 0.05 g of faeces were excluded from data analysis.

2.5.2. Corticosterone/testosterone

2.5.2.1. Blood sampling. At day 88 ± 2, the animals were anaesthetized (Forene, Abbott GmbH, Wiesbaden, Germany) and decapitated immediately after the last confrontation between 8.00 a.m. and 10.00 a.m. Trunk blood was collected in heparinised capillaries. After separation of cellular constitutions by centrifugation (5 min at 13,000 rpm), plasma was frozen at −20 °C until analysis. To avoid stress effects of the handling procedures on the investigated endocrine parameters, blood sampling was performed within at most 3 min after stopping the 10 min of testing [24,30,82].

2.5.2.2. Hormone analysis. For the analysis of plasma corticosterone concentrations, blood samples were analysed using an established DEMEDITEC Enzyme Immunoassay Kit (EIA, DE4164, Demeditec Diagnostics GmbH, Kiel, Germany). All standards, samples, and controls were run in duplicate concurrently. Samples were diluted 1:5, while controls were diluted 1:50. The intra- and inter-assay coefficients of variation for the corticosterone analysis were 3.3% and 6.0%, respectively. Results were only accepted if within the range of 1.44–69.17 ng/ml.

To determine plasma testosterone concentrations, blood samples were analysed using an established DEMEDITEC Enzyme Immunoassay Kit (EIA, DE1559, Demeditec Diagnostics GmbH, Kiel, Germany). All standards, samples, and controls were run in duplicate concurrently. Samples as well as controls were not diluted. The intra- and inter-assay coefficients of variation for the testosterone were 5.7% and 7.2%, respectively. Samples with testosterone concentrations <0.2 ng/ml were excluded from further analysis.

2.5.3. Adrenal tyrosine hydroxylase (TH)

Adrenal glands were dissected, transferred to a 1.5 ml reaction tube containing Tris–HCl buffer (pH 7.2), quick-frozen and then stored at −70 °C. For analysis of the TH activity, the adrenals were gently defrosted and homogenized in 150 μl 5 mM Tris–HCl buffer (pH 7.2). After centrifugation (14,000 rpm) for 30 min at 4 °C, TH was determined in the supernatant by means of a radioenzymatic method according to the method of [64] with slight modifications as described in ([90]; see also [38]).

2.6. Statistical analysis

All data sets were checked for normal distribution by a descriptive analysis of the histogram as well as by the Kolmogorov–Smirnov test. If data did not significantly deviate from a normal distribution, it was analysed using a two-way ANOVA with *genotype* and *environmental situation* as between subject factors. In cases of significance sequential Bonferroni-corrected independent-samples *t*-tests followed.

If data was not normally distributed and could not be transformed to a normal distribution, non-parametric statistical tests were used. To analyse three independent samples, the Kruskal–Wallis *H*-test was performed. In cases of significance, two independent samples were compared using the Mann–Whitney *U*-test. In cases of multiple Mann–Whitney *U*-tests, subsequent sequential Bonferroni-correction was performed.

For analysis of corticosterone metabolites a Repeated Measures ANOVA was performed with *genotype* and *environmental situation* as independent factors, followed by sequential Bonferroni corrected paired samples *t*-tests. Statistical significance was set at $p < 0.05$. All tests were calculated using the software package SPSS (SPSS for Windows, Release 11.5.0., 2002).

3. Results

3.1. Agonistic behaviour (confrontation I)

In all cases, focal animals approached the opponent, indicating their social interest. Afterwards, their behaviour changed into offensive aggression. Therefore, we first present frequencies of *approaching*, followed by the offensive aggressive behaviour (*latency to attack* and *sum of offensive aggressive behaviour*). After that, we describe the defensive behaviour of wildtype, heterozygous, and homozygous 5-HTT knockout males. Finally, the docile C3H opponents are characterised. To increase the readability of this paper only *p*-values of the main effects are presented in the text, wheras *F*-values and χ^2-values are given in Table 2.

3.1.1. Males of all three 5-HTT genotypes

3.1.1.1. Social interest. Concerning the frequency of *approaching* (Fig. 1), no significant main effect of the 5-HTT genotype was detectable ($p = 0.348$). Nonetheless, the ANOVA revealed a main effect of the environmental situation ($p < 0.001$), which is being confronted with an opponent either in the own territory, in the opponent's territory or in a neutral area. Post-hoc analyses showed significantly lower levels of approaching in the opponent's territory for wildtype and homozygous 5-HTT knockout mice compared to the own territory ($t = 2.208/t = 3.806$; $p = 0.038/p = 0.001$) and the neutral area ($t = 5.172/t = 5.412$; both $p < 0.001$). In addition, a significant interaction of the environmental situation and the 5-HTT genotype was found ($p = 0.006$). While heterozygous indi-

Table 2
Confrontation I: effects of genotype (G) and environmental situation (E) as well as gene by environment interaction ($G \times E$).

Behaviour	Effect of Genotype (G)	Environmental situation (E)	$G \times E$
Approaching	$F(2,102) = 1.067$; $p = 0.348$	$F(2,102) = 15.372$; $p < 0.001$	$F(4,102) = 3.887$; $p = 0.006$
Latency to attack	$F(2,102) = 9.683$; $p < 0.001$	$F(2,102) = 1.419$; $p = 0.247$	$F(4,102) = 1.053$; $p = 0.384$
Sum of offensive aggressive behaviour	$F(2,102) = 3.467$; $p = 0.035$	$F(2,102) = 8.417$; $p < 0.001$	$F(4,102) = 2.996$; $p = 0.022$
Following*	$\chi^2 = 0.293$; df = 2; $p = 0.864$	$\chi^2 = 35.532$; df = 2; $p < 0.001$	–
Chasing*	$\chi^2 = 3.325$; df = 2; $p = 0.190$	$\chi^2 = 10.062$; df = 2; $p = 0.007$	–
Attack*	$F(2,102) = 5.299$; $p = 0.006$	$F(2,102) = 2.711$; $p = 0.071$	$F(4,102) = 1.509$; $p = 0.205$
Bite Attack*	$\chi^2 = 1.203$; df = 2; $p = 0.548$	$\chi^2 = 3.297$; df = 2; $p = 0.192$	–
Escalated fighting*	$F(2,102) = 4.543$; $p = 0.013$	$F(2,102) = 0.735$; $p = 0.482$	$F(4,102) = 1.665$; $p = 0.164$
Defensive upright posture	$\chi^2 = 0.423$; df = 2; $p = 0.809$	$\chi^2 = 8.694$; df = 2; $p = 0.013$	–
Defensive sidewise posture	$\chi^2 = 0.013$; df = 2; $p = 0.994$	$\chi^2 = 20.234$; df = 2; $p < 0.001$	–
Attack initiated by C3H	$\chi^2 = 1.428$; df = 2; $p = 0.490$	$\chi^2 = 34.658$; df = 2; $p < 0.001$	–
Bite Attack initiated by C3H	$\chi^2 = 0.175$; df = 2; $p = 0.916$	$\chi^2 = 10.012$; df = 2; $p = 0.007$	–
Escalated fighting initiated by C3H	$\chi^2 = 0.577$; df = 2; $p = 0.749$	$\chi^2 = 22.766$; df = 2; $p < 0.001$	–

Statistics: ANOVA, Kruskal-Wallis H-test; Grey: $p < 0.05$; *: Following, Chasing, Attack, Bite Attack and Escalated fighting are included in the parameter *sum of offensive aggressive behaviour*. *Note*: In case of non-parametric statistics no $G \times E$ interactions can be given.

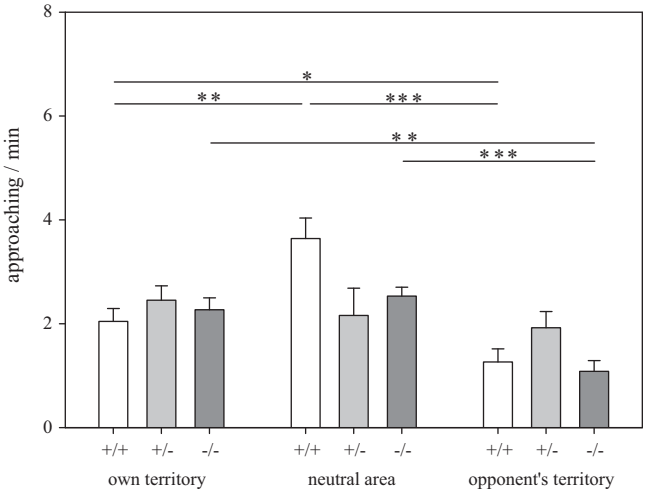

Fig. 1. Social interest (approaching). Data are shown as mean + SEM. +/+, 5-HTT wildtype mice; +/−, heterozygous 5-HTT knockout mice; −/−, homozygous 5-HTT knockout mice. Sample sizes: $N = 11$–14; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

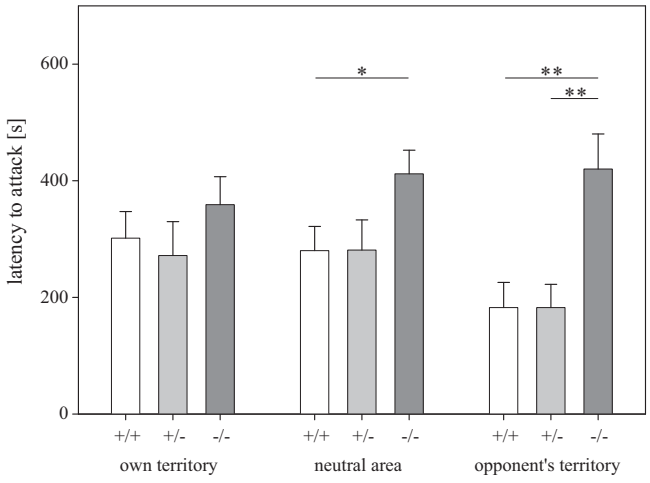

Fig. 2. Latency to attack. Data are shown as mean + SEM. +/+, 5-HTT wildtype mice; +/−, heterozygous 5-HTT knockout mice; −/−, homozygous 5-HTT knockout mice. Sample sizes: $N = 11$–14; *: $p < 0.05$; **: $p < 0.01$.

viduals did not differ between the three environmental situations, the effects for wildtype and homozygous knockout mice were as already described.

3.1.1.2. Offensive aggressive behaviour. Concerning the *latency to attack* the opponent (Fig. 2), the ANOVA revealed a main effect of genotype ($p < 0.001$). Post-hoc analyses showed that this effect could only be found in the opponent's territory and in the neutral area. In the opponent's territory, homozygous 5-HTT knockout mice displayed significantly longer attack latencies than wildtype and heterozygous 5-HTT knockout mice ($t = -3.311$/$t = -3.299$; both $p = 0.004$). In the neutral area, the attack latency of the 5-HTT−/− mice was longer, too, but this effect was only significant in comparison with wildtypes ($t = -2.244$; $p = 0.035$). Interestingly, no differences between the genotypes could be found in the own territory.

The *sum of offensive aggressive behaviour* consists of the added frequencies of the behavioural patterns *attack*, *following*, *escalated fighting*, *chasing* and *bite attack* (for a separate statistical analysis see Table 2). Concerning the *sum of offensive aggressive behaviour* (Fig. 3), a significant main effect of the 5-HTT genotype was detectable ($p = 0.035$). Post hoc analyses indicated that in their own territory, heterozygous 5-HTT knockout mice were more aggressive than wildtypes ($t = -2.435$; $p = 0.028$), while levels of aggression in homozygous 5-HTT knockout mice lay in between.

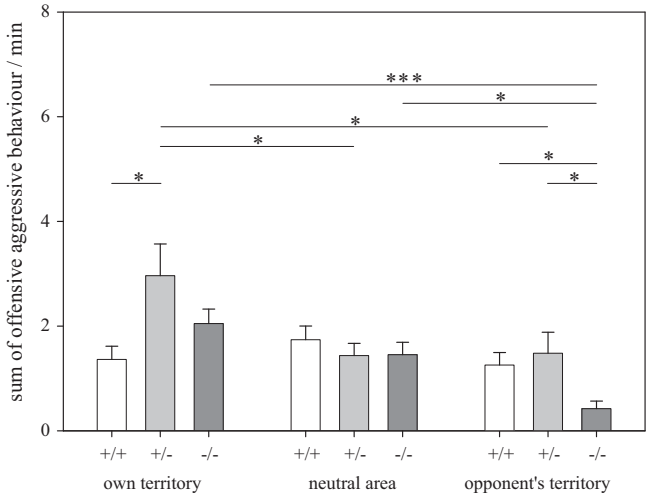

Fig. 3. Sum of offensive aggressive behaviour (following, chasing, attack, bite attack, and escalated fighting). Data are shown as mean + SEM. +/+, 5-HTT wildtype mice; +/−, heterozygous 5-HTT knockout mice; −/−, homozygous 5-HTT knockout mice. Sample sizes: $N = 11$–14; *: $p < 0.05$; ***: $p < 0.001$.

In the opponent's territory, homozygous 5-HTT knockout mice were less aggressive than wildtypes and heterozygous knockout mice ($t = 2.976/t = 2.489$; $p = 0.007/p = 0.025$). In the neutral area, no differences between the genotypes could be found. The ANOVA revealed also a significant main effect of the environmental situation ($p < 0.001$) on the sum of offensive aggressive behaviour. Post hoc analyses indicated more offensive aggressive behaviour for heterozygous and homozygous 5-HTT knockout mice in the own territory than in the opponent's territory ($t = 2.071/t = 5.238$; $p = 0.050/p < 0.001$). Heterozygous mice also showed more aggressive behaviour in their own territory than in the neutral area ($t = 2.350$; $p = 0.034$). Finally, homozygous 5-HTT knockout mice were less aggressive in the opponent's territory than in the neutral area ($t = 3.558$; $p = 0.002$). In addition, a significant interaction of the environmental situation and the 5-HTT genotype was found ($p = 0.022$). While heterozygous and homozygous 5-HTT knockout mice differed concerning their levels of offensive aggressive behaviour between the three environmental situations, the frequency of aggressive behaviour of wildtype mice did not vary significantly, irrespective of whether the confrontation took place in their own or in the opponent's territory or in the neutral area.

3.1.1.3. Defensive behaviour. In general, very few defensive postures were displayed by the focal mice. Nevertheless, the analyses of the *defensive upright posture* and *defensive sidewise posture* (Table 2) revealed a significant main effect of the environmental situation ($p = 0.013/p < 0.001$) with most defensive behaviour being performed in the opponent's territory. There was no main effect of genotype and no significant gene by environment interaction.

3.1.2. C3H males

Attacks initiated by C3H males (Fig. 4) rarely occurred and were environment dependent ($p < 0.001$). Post hoc analyses indicated that significantly more attacks were performed towards heterozygous and homozygous 5-HTT knockout mice in the opponent's territory (home cage of the C3H mice) compared with the own territory of the focal animals ($U = 18.500/U = 14.500$; $p = 0.001/p < 0.001$). Similarly, the C3H males showed more attacks towards homozygous 5-HTT knockout males in the opponent's territory than in the neutral area ($U = 7.000$; $p < 0.001$). Interestingly, the frequency of C3H attacks towards 5-HTT wildtype mice did not differ between the three environmental situations. Additionally, no main effect of the 5-HTT genotype was found ($p = 0.490$). These results are consistent with the data for the behavioural patterns *bite attack initiated by C3H male* and *escalated fighting initiated by C3H male* which can be seen in Table 2.

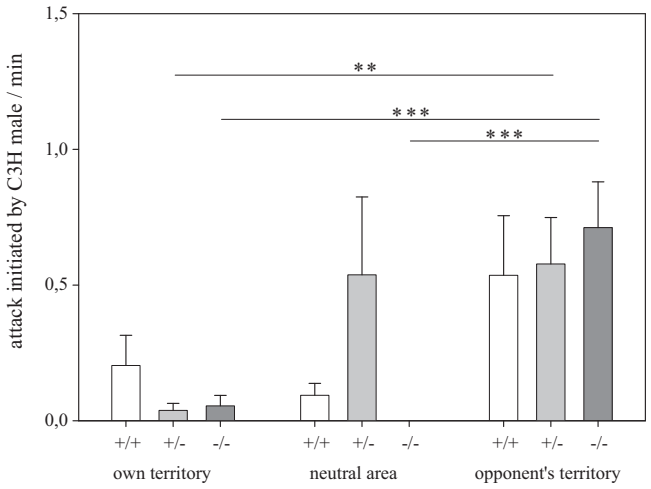

Fig. 4. Attack initiated by C3H male. Data are shown as mean + SEM. +/+, 5-HTT wildtype mice; +/−, heterozygous 5-HTT knockout mice; −/−, homozygous 5-HTT knockout mice. Sample sizes: $N = 11–14$; **: $p < 0.01$; ***: $p < 0.001$.

3.2. Agonistic behaviour (confrontations II, and III)

The analysis of the behaviour of wildtype and 5-HTT knockout mice as well as of C3H males in a second and third confrontation revealed that the pattern of differences of main effects concerning genotype and environmental situation found in the first confrontation persisted over time. To enhance the readability of this paper, we will only refer to the main effects of the analysis and give the *p*-values in the text. *F*-values and and χ^2-values are given in Tables 3 and 4.

3.2.1. Males of all three 5-HTT genotypes

3.2.1.1. Social interest. Concerning *approaching*, the significant main effect of the environmental situation detected in the first confrontation persisted also in the second and third one (both: $p < 0.001$). In contrast, the significant interaction of the 5-HTT genotype and the environmental situation in the first confrontation was shown only by a trend in confrontation 2 ($p = 0.095$), and it was not detectable in the third one at all ($p = 0.717$).

3.2.1.2. Offensive aggressive behaviour. The analysis of the *latency to attack* in the second confrontation revealed a main effect of the 5-HTT genotype ($p = 0.013$) that was also found in the first confrontation. In confrontation II, an effect of the environmental situation was detected that was absent in the first trial ($p < 0.001$).

Table 3
Confrontation II: Effects of genotype (G) and environmental situation (E) as well as gene by environment interaction ($G \times E$).

Behaviour	Effect of Genotype (G)	Environmental situation (E)	$G \times E$
Approaching	$F(2,99) = 0.978$; $p = 0.380$	$F(2,99) = 27.440$; $p < 0.001$	$F(4,99) = 2.037$; $p = 0.095$
Latency to attack	$F(2,99) = 4.534$; $p = 0.013$	$F(2,99) = 9.023$; $p < 0.001$	$F(4,99) = 1.255$; $p = 0.293$
Sum of offensive aggressive behaviour	$F(2,99) = 2.676$; $p = 0.074$	$F(2,99) = 11.020$; $p < 0.001$	$F(4,99) = 0.765$; $p = 0.551$
Following*	$\chi^2 = 0.070$; df = 2; $p = 0.966$	$\chi^2 = 21.788$; df = 2; $p < 0.001$	–
Chasing*	$\chi^2 = 6.080$; df = 2; $p = 0.048$	$\chi^2 = 3.810$; df = 2; $p = 0.149$	–
Attack*	$F(2,99) = 2.419$; $p = 0.094$	$F(2,99) = 6.099$; $p = 0.003$	$F(4,99) = 0.922$; $p = 0.454$
Bite Attack*	$\chi^2 = 3.883$; df = 2; $p = 0.144$	$\chi^2 = 6.777$; df = 2; $p = 0.034$	–
Escalated fighting*	$\chi^2 = 2.896$; df = 2; $p = 0.235$	$\chi^2 = 18.702$; df = 2; $p < 0.001$	–
Defensive upright posture	$\chi^2 = 0.364$; df = 2; $p = 0.834$	$\chi^2 = 33.036$; df = 2; $p < 0.001$	–
Defensive sidewise posture	$\chi^2 = 0.737$; df = 2; $p = 0.692$	$\chi^2 = 23.717$; df = 2; $p < 0.001$	–
Attack initiated by C3H	$\chi^2 = 4.956$; df = 2; $p = 0.084$	$\chi^2 = 34.605$; df = 2; $p < 0.001$	–
Bite Attack initiated by C3H	$\chi^2 = 5.823$; df = 2; $p = 0.054$	$\chi^2 = 11.702$; df = 2; $p = 0.003$	–
Escalated fighting initiated by C3H	$\chi^2 = 5.275$; df = 2; $p = 0.072$	$\chi^2 = 18.183$; df = 2; $p < 0.001$	–

Statistics: ANOVA, Kruskal-Wallis *H*-test; Grey: $p < 0.05$; *: Following, Chasing, Attack, Bite Attack and Escalated fighting are included in the parameter *sum of offensive aggressive behaviour*. *Note*: In case of non-parametric statistics no $G \times E$ interactions can be given.

Table 4
Confrontation III: effects of genotype (G) and environmental situation (E) as well as gene by environment interaction ($G \times E$).

Behaviour	Effect of Genotype (G)	Environmental situation (E)	$G \times E$
Approaching	$F(2,92) = 1.572$; $p = 0.213$	$F(2,92) = 19.658$; $p < 0.001$	$F(4,92) = 0.525$; $p = 0.717$
Latency to attack	Not determined	Not determined	Not determined
Sum of offensive aggressive behaviour	$F(2,92) = 4.818$; $p = 0.010$	$F(2,92) = 5.020$; $p = 0.009$	$F(4,92) = 0.947$; $p = 0.440$
Following*	$\chi^2 = 1.929$; df = 2; $p = 0.381$	$\chi^2 = 18.740$; df = 2; $p < 0.001$	–
Chasing*	$\chi^2 = 4.643$; df = 2; $p = 0.098$	$\chi^2 = 3.432$; df = 2; $p = 0.180$	–
Attack*	$\chi^2 = 8.524$; df = 2; $p = 0.014$	$\chi^2 = 6.532$; df = 2; $p = 0.038$	–
Bite Attack*	$\chi^2 = 3.937$; df = 2; $p = 0.140$	$\chi^2 = 11.692$; df = 2; $p = 0.003$	–
Escalated fighting*	$\chi^2 = 9.395$; df = 2; $p = 0.009$	$\chi^2 = 2.872$; df = 2; $p = 0.238$	–
Defensive upright posture	$\chi^2 = 0.452$; df = 2; $p = 0.798$	$\chi^2 = 12.604$; df = 2; $p = 0.002$	–
Defensive sidewise posture	$\chi^2 = 0.005$; df = 2; $p = 0.997$	$\chi^2 = 7.872$; df = 2; $p = 0.020$	–
Attack initiated by C3H	$\chi^2 = 2.724$; df = 2; $p = 0.422$	$\chi^2 = 8.741$; df = 2; $p = 0.006$	–
Bite Attack initiated by C3H	$\chi^2 = 0.953$; df = 2; $p = 0.621$	$\chi^2 = 5.195$; df = 2; $p = 0.074$	–
Escalated fighting initiated by C3H	$\chi^2 = 2.880$; df = 2; $p = 0.866$	$\chi^2 = 0.130$; df = 2; $p = 0.937$	–

Statistics: ANOVA, Kruskal-Wallis H-test; Grey: $p < 0.05$; *: Following, Chasing, Attack, Bite Attack and Escalated fighting are included in the parameter *sum of offensive aggressive behaviour*. *Note*: In case of non-parametric statistics no $G \times E$ interactions can be given.

In both confrontations, no genotype by environment interaction existed ($p = 0.293$).

The significant main effect of the 5-HTT genotype for *the sum of offensive aggressive behaviour* in the first confrontation was confirmed in the third confrontation ($p = 0.01$) and is supported by a trend in the second one ($p = 0.074$). Additionally, the main effect of the environmental situation in the first confrontation was confirmed in confrontation 2 and 3 ($p < 0.001/p = 0.009$).

3.2.1.3. Defensive behaviour. The analysis of the *defensive upright posture* in the second and third confrontation was in line with the first one: although the environmental situation influenced the behavioural patterns significantly ($p < 0.001/p = 0.002$), no effect of genotype was detectable ($p = 0.834/p = 0.798$).

3.2.2. C3H males

C3H males behaved rather consistently in all three confrontations and were not influenced by the 5-HTT genotype but by the environmental situation (Tables 3 and 4).

3.3. Endocrinological investigations

3.3.1. Corticosterone metabolites (CM)

The analysis of faecal samples collected before the first and after the second confrontation with a conspecific revealed a significant main effect of confrontation, that is, CM concentrations were higher after repeated confrontations with a conspecific ($F(1,100) = 951.009$, $p < 0.001$; Fig. 5A). Post-hoc comparisons showed that wildtype mice confronted with a C3H male in the own as well as in the opponent's territory displayed a significant increase in CM concentrations ($t = -2.788/t = -2.579$; $p = 0.018/p = 0.027$). In heterozygous mice, this effect was only detectable in the neutral area ($t = -2.822$; $p = 0.017$). However, in homozygous 5-HTT knockout mice, no significant increase in CM concentrations was detectable ($t = -0.364/t = -1.974/t = -0.832$; $p = 0.722/p = 0.072/p = 0.423$). Additionally, the Repeated Measures ANOVA detected no effect of genotype, environmental situation or an interaction of these two parameters (*environmental situation*: $F(2,100) = 0.779$, $p = 0.462$; *genotype*: $F(2,100) = 0.131$, $p = 0.878$; *environmental situation*genotype*: $F(2,100) = 0.515$, $p = 0.725$).

3.3.2. Plasma corticosterone (CORT)

The analysis of CORT concentrations immediately after the last confrontation revealed a main effect of the environmental situation ($F(2,102) = 13.453$, $p < 0.001$; Fig. 5B) with lowest stress hormone concentrations in the own territory. In wildtype and heterozygous 5-HTT knockout mice, significantly higher CORT concentrations were found in the neutral area than in their own territories ($t = -3.159/t = -2.672$; $p = 0.005/p = 0.016$). Additionally, wildtypes showed significant higher CORT concentrations in the neutral area than in the opponent's territory ($t = 2.529$; $p = 0.019$). Strikingly, for homozygous 5-HTT knockout mice, CORT concentrations were nearly the same in all three environmental situations ($t = -1.767/t = -2.046/t = -0.223$; $p = 0.093/p = 0.058/p = 0.825$).

3.3.3. Plasma testosterone (TEST)

The analysis of TEST concentrations immediately after the last confrontation revealed no effects of the environmental situation ($F(2,98) = 0.272$, $p = 0.762$; Fig. 6) or the 5-HTT genotype ($F(2,98) = 2.081$, $p = 0.130$). Also, no interaction between these two parameters was detectable ($F(4,98) = 1.850$, $p = 0.125$).

3.3.4. Adrenal tyrosine hydroxylase (TH)

The analysis of TH activity immediately after the last confrontation revealed a main effect of genotype ($F(2,71) = 5.714$, $p = 0.005$; Fig. 7), with higher values in heterozygous 5-HTT knockout mice in all three environmental situations. Post-hoc analysis revealed that TH activity was significantly different between heterozygous and homozygous mice in the neutral area and the opponent's territory ($t = 2.324/t = 2.381/t = 0.037$; $p = 0.031$). However, this effect did not remain significant after Bonferroni correction. Neither an effect of the environmental situation ($p = 0.850$) nor a gene by environment interaction ($p = 0.840$) was detectable.

4. Discussion

The overall aim of the present study was to investigate how the 5-HTT genotype and the environmental situation in which a confrontation takes place shape the display of offensive aggressive behaviour. To enhance the motivation of the focal animals to perform offensive aggression as well as to avoid a domination of their behaviour by the opponent, males of the docile strain C3H were chosen as contestants. Indeed, C3H mice displayed overall very low levels of aggressive behaviour towards the focal individuals that were comparable between mice of all three 5-HTT genotypes. Accordingly, wildtype, heterozygous, and homozygous 5-HTT knockout mice very rarely displayed defensive behaviour towards the opponents. Using this design, we identified three different phases of the contest situation: The first was the phase of information gathering that depended on the environmental situation, but not on the individual's genotype. In the second phase, mice decided how quickly to attack the opponent, which depended on the genotype, but not on the environmental situation. The third phase was the physical interaction, which was influenced by the

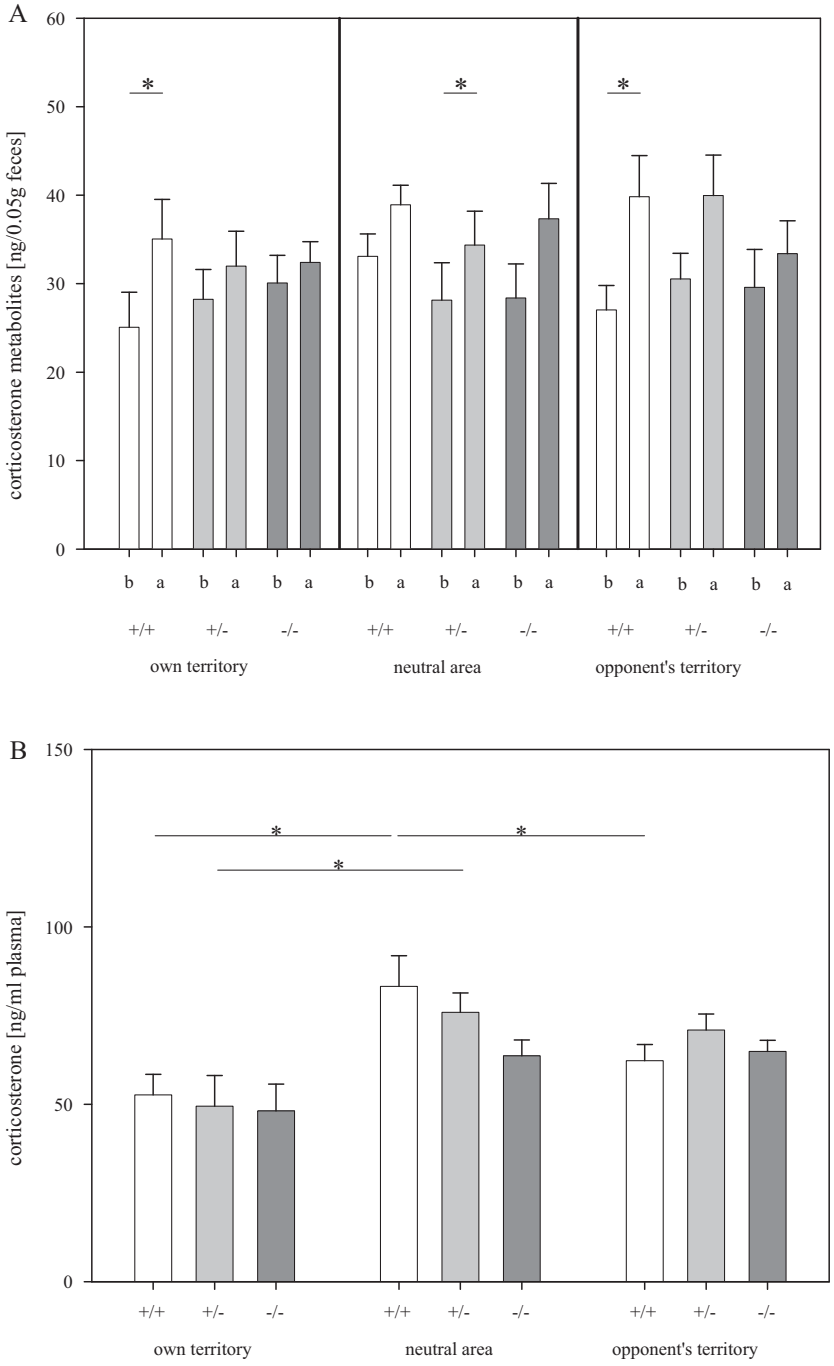

Fig. 5. Activity of the hypothalamic-pituitary-adrenocortical (HPA) axis. Concentrations of corticosterone metabolites in the faeces (A) as well as plasma corticosterone concentrations (B) were analysed. Data are shown as mean + SEM. +/+, 5-HTT wildtype mice; +/−, heterozygous 5-HTT knockout mice; −/−, homozygous 5-HTT knockout mice. b, before confrontation; a, after confrontation. Sample sizes: $N = 11–14$; *: $p < 0.05$.

5-HTT genotype, the environmental situation and a complex interplay of genotype and environment. A comparison of data from the three confrontations showed that these observed effects were predominantly stable and consistent over time.

4.1. Behaviour

4.1.1. Information gathering

Irrespective of their genotypes, all focal animals showed nearly the same frequency of *approaching* the contestant in all three environmental situations, thereby gaining olfactory, auditory, and tactile information about him [60,63]. This stands in line with data from other studies finding also no differences in social interest behaviour and social exploration between the three genotypes ([31,49]; but, see also: [39,61]). In contrast, levels of social interest behaviour strongly depended on the environmental situation in which a contest took place. Wildtypes and homozygous 5-HTT knockout mice approached the contestant less frequently in the opponent's territory compared with the own territory and the neutral area, which is in general not surprising for territorial mammals like mice [52,71,73]. A novel environment with an unfamiliar male opponent means a potential risk for the intruding individual of being attacked and injured by the territory holder. Being cautious and approach the opponent less frequently can therefore minimize

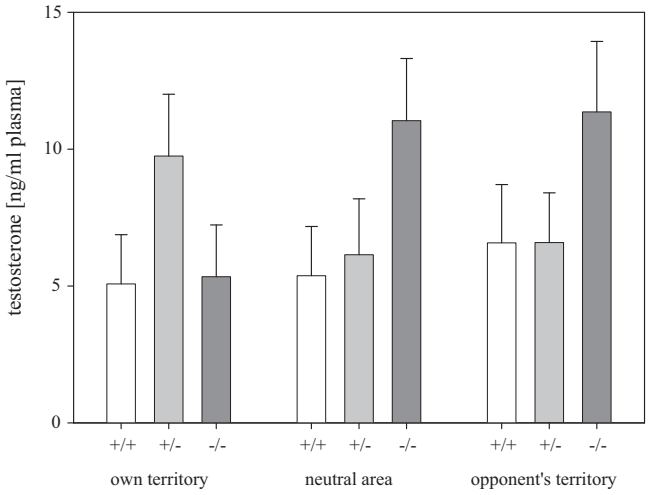

Fig. 6. Plasma testosterone concentrations. Data are shown as mean + SEM. +/+, 5-HTT wildtype mice; +/−, heterozygous 5-HTT knockout mice; −/−, homozygous 5-HTT knockout mice. Sample sizes: $N = 9–14$.

the potential costs of fighting [34]. In addition, the own territory has a probably high yield of food or mates, making it reasonable from a socio-biological perspective to provide most energy for its defence [23,70]. To be most effective in doing so, one should be alert right from the beginning of a contest situation, a fact that is reflected here by the higher frequency of *approaching* in the own territory.

4.1.2. Decision how quickly to attack the opponent

For the *latency to attack* the opponent, a significant main effect of the 5-HTT genotype was found. In particular, homozygous 5-HTT knockout mice showed significantly longer attack latencies than wildtypes and heterozygotes when confronted with a C3H male in both, the neutral area and the opponent's territory, thereby confirming hypothesis 1. Strikingly, in the own territory, no differences between the genotypes were detected at all.

Behavioural ecological models include assumptions about the level of information a contestant has about the costs (C) and benefits (B) of possible behavioural options in a contest [34]. Of particular relevance for the interpretation of behavioural data presented here is that the behaviour in a contest can be influenced by the individual's *perception* of these costs [34]. If potential fighting costs [25,27,65] are perceived as rather high, e.g. in terms of the probability of injury, individuals should retreat from a contest sooner or should not engage in it at all (reviewed in: [34]). That means, the B/C ratio is relatively low and accordingly, the threshold for eliciting aggressive behaviour is high [65]. In contrast, if potential fighting costs are perceived as rather low (higher B/C ratio), individuals should engage in a contest situation or prolong it (reviewed in: [34]). In such a situation, the threshold for eliciting aggressive behaviour is relatively low. It seems reasonable to assume that the perception of fighting costs is influenced by individual levels of anxiety. That means: Individuals with generally higher levels of anxiety, as it is the case in 5-HTT knockout mice [29,32,36], tend to perceive the potential fighting costs as higher than individuals with lower levels of anxiety, leading to a higher threshold for eliciting aggressive behaviour and consequently to significantly longer attack latencies. If a homozygous 5-HTT knockout mouse is placed into an unfamiliar environment (neutral area and opponent's territory), this probably intensifies the inborn differences in anxiety. Additionally, in the opponent's territory, the behaviour of the C3H mice was a little more offensive than in the other environmental situations (indicated by a statistical main effect), which probably enhanced levels of anxiety, too. Consistently, homozygous 5-HTT knockout mice showed significantly longer attack latencies in the neutral area and opponent's territory compared with wildtypes and heterozygotes. Interestingly, being in the own territory seemed to compensate these differences in anxiety, probably because of a stress-buffering effect of the familiar environment ([89]; see Section 4.2), and caused the homozygous 5-HTT knockout mice to behave in nearly the same way as wildtypes and heterozygotes.

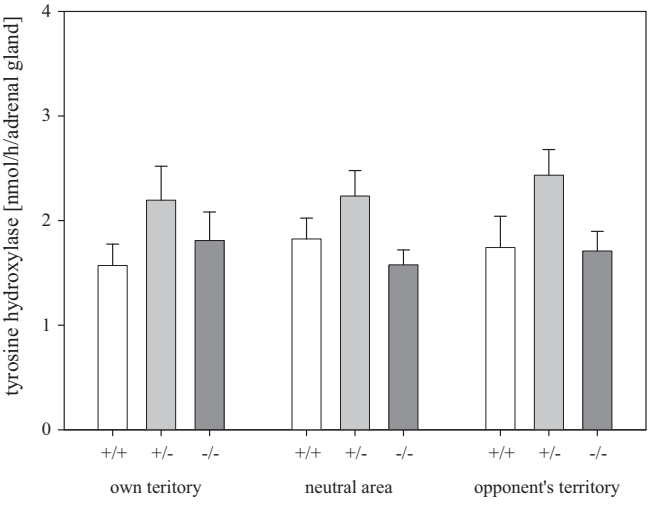

Fig. 7. Tyrosine hydroxylase activity. Data are shown as mean + SEM. +/+, 5-HTT wildtype mice; +/−, heterozygous 5-HTT knockout mice; −/−, homozygous 5-HTT knockout mice. Sample sizes: $N = 6–11$.

Holmes et al. [31] found longer attack latencies in homozygous 5-HTT knockout mice compared to wildtype controls when the mice were confronted in their own home cages with an intruding conspecific. At first glance, this finding stands in contrast to our data. It is known, however, that the behaviour in an aggressive encounter depends on the type of opponent [10,21,55,57]. Therefore, the named differences are probably due to the fact that Holmes et al. [31] used opponents from a more aggressive strain (DBA/2J) than we did [37] inducing more anxiety in the homozygous 5-HTT knockout mice and thus increasing the threshold for aggressive behaviour.

4.1.3. Physical interaction

The sum of offensive aggressive behaviour was influenced by the 5-HTT genotype and the environmental situation as well as by a genotype by environment interaction. Concerning genotype, it was found that homozygous 5-HTT knockout mice performed significantly less offensive aggressive behaviour in the opponent's territory compared with wildtype and heterozygous mice, which confirms hypothesis 1. These results stand in line with the discussion about differences in inborn levels of anxiety. Additionally, one cannot exclude that genotype-dependent variations in levels of aggression were also influenced by a differential response to the factor single housing [57].

Concerning the environmental situation, generally less offensive aggression was displayed outside the own territory, thereby underlying the discussion about socio-biological reasons presented before and confirming hypothesis 2.

For the discussion of our results, the genotype by environment interaction is of particular interest. In general, such an interaction means that a genotype effect is influenced by the environmental situation or, vice versa, that the influence of an environmental situation is modified by genotype. Several studies in 5-HTT knockout mice show that genotype by environment interactions are essentially involved in the display of anxiety-like behaviour [14,29,36]. In the present study, we demonstrate for the first time that geno-

type by environment interactions are also crucial for bringing about offensive aggressive behaviour: In sum, homozygous and heterozygous 5-HTT knockout mice displayed less offensive aggression outside the own territory, whereas wildtypes behaved nearly the same in all three environmental situations.

A central insight derived from the present study is: The level of offensive aggression displayed is influenced by the inborn level of anxiety and the anxiety-inducing effect of the environmental situation. As a consequence, mice of the three 5-HTT genotypes differed in their decision rules for the display of offensive aggression towards a docile opponent: In wildtypes, the decision to perform offensive aggression does not seem to be influenced by environmental cues. Irrespective of the environmental situation in which a contest took place or of the opponent's behaviour, they displayed nearly the same level of offensive aggression. In heterozygous mice, the decision rule depends on being in the own territory or in an unfamiliar environment. They showed highest amounts of offensive aggressive behaviour in the own territory. Aggression, however, did not differ between the neutral area and the opponent's territory. In homozygous 5-HTT knockout mice, the decision to display offensive aggression is influenced by both the environmental situation and the opponent's behaviour. Thus, highest amounts of aggression were displayed in the own, lowest levels in the opponent's territory. In the neutral area an intermediate amount of offensive aggression was found.

We do not assume one decision rule to be better than the other, but rather think them to be context-dependent. In future studies, it will therefore be especially interesting to elucidate which decision rule is advantageous in which environmental condition.

4.2. Endocrinology

The two major components of the stress system are the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympathetic–adrenomedullary system (SAS) [4,11,86]. The activation of each of these systems plays a major role in adjusting an individual to social and non-social stressors by providing the organism with energy and shifting it into a state of heightened reactivity [77].

Concerning the HPA axis, we monitored glucocorticoid secretion before the first and after the second confrontation non-invasively by measuring faecal corticosterone metabolites (CM). Consistent with numerous findings in a variety of species [74,78,86], the statistical main effect of the confrontation itself indicated that CM concentrations increased significantly due to repeated fighting irrespective of genotype or environmental situation. The magnitude of the stress response seems to vary with certain environmental circumstances as shown by the corticosterone concentrations from blood samples (CORT) taken immediately after the last confrontation. Namely, the data revealed a significant main effect of the environmental situation, generally with lowest levels of stress in the own territory of the focal animals, thereby confirming hypothesis 3. This finding might be due to some kind of stress buffering effect by the familiar environment [89]. The fact that no influence of the 5-HTT genotype on CM and CORT concentrations was found stands in line with findings of Jansen et al. [36] and Tjurmina et al. ([83]; but, see also: [43,50]). The difference in CM and CORT concentrations concerning the role of the environmental situation on stress hormone secretion might be due to the differences in time point of sampling and the sampling intervals.

Concerning the SAS, we investigated tyrosine hydroxylase (TH) activity after the last confrontation and found a significant main effect of the 5-HTT genotype with generally highest levels in heterozygous 5-HTT knockout mice. This is consistent with hypothesis 5 and might, in combination with other factors, explain the high levels of offensive aggression in heterozygous 5-HTT knockout mice in their own territory (see Section 4.3). It is known that 5-HT can influence catecholamine release by direct actions in the adrenal medulla [83]. Furthermore, 5-HTT binding sites have been identified in the rat adrenal medulla, and epinephrine (EPI)-synthesizing cells accumulate and store 5-HT via the 5-HTT and the vesicular monoamine transporter [79]. A study by Tjurmina et al. [83] investigated the activity of the SAS in the three 5-HTT genotypes as well and pointed towards an effect of genotype with homozygous 5-HTT knockout mice displaying the highest EPI concentrations after immobilisation stress. At first sight, these results seem to be contradictory to the data of the present study. However, whereas EPI indicates the acute response to a given stressor, TH activity rather reflects the basal activity of the animals' SAS [18,26,38,76,77].

Testosterone (TEST) is an important mediator of aggressive behaviour [13,16,23,51,66,74]. Surprisingly, the analysis of TEST concentrations after the last confrontation revealed neither an effect of the 5-HTT genotype or the environmental situation nor an interaction of these two factors, which contradicts hypothesis 4. This is possibly due to two reasons. Firstly, blood sampling was performed within about 10 min after the beginning of the last confrontation. Maybe a longer time is required to detect a significant change in TEST concentrations. Secondly, the confrontation of wildtype, heterozygous, and homozygous 5-HTT knockout mice with a docile C3H opponent possibly led to some kind of winner experience in all focal animals. Winning is known to increase TEST concentrations in many vertebrates [33,69,74]. Probably, an increase in TEST concentration led to the same result in all three environmental situations, therefore making it impossible to detect any differences in these hormone concentrations after the last of three aggressive encounters. Thus, possible differences in basal TEST concentrations between the genotypes may be concealed.

4.3. Risk profile for the display of inadequately high aggression

There have been attempts to identify risk factors causing high aggression, antisocial behaviour, and violence (for review see [3]). Arregi et al. [3], for example, assume the combination of dominance, high serum androgen concentrations, low adrenocortical activity, and a reduced serotonergic activity in the CNS to be a critical risk profile, making the display of offensive aggression likely. In the present study, heterozygous 5-HTT knockout mice displayed in their own territory a level of offensive aggressive behaviour towards the docile opponents that is, from a socio-biological perspective, in its intensity a waste of time and energy [80]. These data point towards a risk profile for exaggeratedly high aggression, because, in accordance with Arregi and colleagues [3], heterozygous 5-HTT knockout mice combine the traits dominance, low adrenocortical acivity as derived from basal CM concentrations before the first confrontation, possibly increased TEST titres (see Section 4.2), and a reduced intraneuronal serotonergic activity [7] in the own territory. Additionally, the present study points to one more key factor that possibly might bring about high aggression scores: a high activity of the SAS reflected by a high activity of the adrenal enzyme tyrosine hydroxylase [76].

5. Conclusion

Our data show that the amount of offensive aggression displayed during a contest depended on the 5-HTT genotype, the environmental situation in which a contest took place, and a complex interaction of 5-HTT genotype and environment. It is likely that, due to their varying genetic predisposition for anxiety, mice of the three genotypes were differentially affected by the aversiveness of the respective venue, which influenced their decision to display offensive aggression. As a consequence, the amount of

aggression shown by homozygous 5-HTT knockout mice was influenced by both the venue and the opponent's behaviour, whereas heterozygotes reacted only to the venue and wildtypes behaved always in the same way, irrespective of venue and opponent. In the own territory, an inadequately high level of aggressive behaviour was shown by heterozygous 5-HTT knockout mice. These animals are characterised by dominance, low serum glucocorticoid concentrations, increased TEST titres, reduced intraneuronal serotonergic activity, and high SAS activity. The combination of these traits may represent a risk profile for inadequately high aggression.

Acknowledgements

This research was supported by a grant from the German Research Foundation (DFG) to Norbert Sachser (Sa 389/10-1).

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