Physiology & Behavior 98 (2009) 411–415

Contents lists available at ScienceDirect

Physiology & Behavior

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

Lack of differential serotonin biosynthesis capacity in genetically selected low and high aggressive mice

Deepa Natarajan *, Sietse F. de Boer, Jaap M. Koolhaas

Department of Behavior Physiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

ARTICLE INFO

Article history:
Received 3 February 2009
Received in revised form 28 May 2009
Accepted 9 July 2009

Keywords:
5-HT
TPH
Aggression
5-HTP
Violence
Genetic selection

ABSTRACT

Reduced brain serotonin (5-HT) activity has been linked to impulsive and violent forms of aggression for decades. Despite a vast accumulation of data pertinent to the above observation, information about the possible mechanisms underlying such a decreased 5-HT functioning is virtually absent. Amongst many, reduced 5-HT biosynthetic capacity is a likely possibility in violent individuals and/or in high-aggressive animals. In order to examine this hypothesis, the current study principally aimed at the determination and comparison of the 5-HT biosynthetic capacity in three different strains of high- and low-aggressive mice obtained by artificial genetic selection. While low Tryptophan Hydroxylase (TPH) activity can be expected to lead to low 5-HT levels and pathological aggression, high TPH activity can be expected to increase 5-HT levels and normal territorial aggression. The above hypothesis was assessed by estimating the *in-vivo* synthesis rate and synthesis rate constant of 5-HT biochemically by measuring the accumulation of 5-hydroxytryptophan (5-HTP) following treatment with the central aromatic amino-acid decarboxylase inhibitor 3-hydroxybenzylhydrazine (NSD-1015). Surprisingly, we found no differences in the 5-HT biosynthetic capacity between the high- and low-aggressive selection lines in their prefrontal cortices and raphe nuclei, two main brain regions closely involved in aggression control. Thus, the underlying inherent genetic differences in aggressiveness observed in these artificially selected mouse strains are not due to constitutive functional differences in their TPH activity in these brain regions.

1. Introduction

The brain 5-HT system has been by far the major focus of neurobiological inquiries into the plausible neurochemical mechanisms mediating aggressive behavior. In animals and humans alike, the most frequently reported finding has been the association of impulsive and excessively aggressive behavioral traits with low central 5-HT neurotransmission activity, generally known as the 5-HT deficiency hypothesis of aggression [4,15]. Very few studies have been performed hitherto to unravel the possible mechanisms underlying such a 5-HT hypofunction. Among the many mechanisms and factors that regulate 5-HT activity in the brain, the 5-HT biosynthetic machinery is likely to play a pivotal role. The key rate-limiting enzyme is Tryptophan hydroxylase (TPH; the TPH_2 particularly is the major neural isoform in the brain) which catalyzes the conversion of the amino-acid tryptophan to 5-hydroxytryptophan (5-HTP). The latter is rapidly decarboxylated further to 5-HT by aromatic-L-aminoacid decarboxylase (AADC) [13].

Evidence for the ability of the brain to utilize TPH enzyme as a regulatory component in the 5-HT control of aggression comes from several lines of research. In particular, genetic and pharmacological approaches have provided clues supportive of a TPH-aggression inverse relationship. Genetic-linkage studies in humans have demonstrated an association of aggression and anger-related traits with functional polymorphisms in the TPH gene that reduce the enzyme's activity or stability [23,27,32]. Genetic-association and -manipulation studies in animals have shown that wild Norway rats and silver foxes selectively bred for low aggressiveness (i.e., docility) towards humans have higher brain TPH activity and hence higher 5-HT levels than their aggressive counterparts [31]. The moderate to high aggressive BALB/cJ mouse strain was found to possess a low functioning TPH_2 allele (1473G homozygote) when compared to the low aggressive C57BL/6J, 129X1/SvJ and A/J strains which possess a high functioning 1473C homozygous TPH_2 allele [12,21]. The TPH2 1473G allele has been reported to result in a 50% reduction of 5-HT biosynthesis rates and brain tissue 5-HT content [33,43]. The Neuropeptide Y $Y1^{-/-}$ mice has been shown to increase their territorial behavior with a decline in the TPH mRNA expression in the CNS [18]. Physical attacks against a non-aggressive male were shown to be enhanced in the homozygous (HO) R439H TPH2 knock-in mice, the latter characterized by vastly reduced (almost 80%) synthesis rate of 5-HTP and reduced tissue content of both 5-HT and its metabolite 5-HIAA [2]. Pharmacological manipulation studies employing the irreversible TPH enzyme inhibitor para-chlorophenylalanine methyl ester hydrochloride (PCPA) that markedly (50–90%) decrease brain 5-HT levels, have been reported to enhance aggressiveness in several studies across various animal species [1,5,10,20,42].

* Corresponding author. Tel.: +31 50 363 2337; fax: +31 50 363 2331.
E-mail address: d.natarajan@rug.nl (D. Natarajan).

0031-9384/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2009.07.009

We recently demonstrated that while the artificially-selected high-aggressive North-Carolina (NC900) and Turku-aggressive (TA) mouse lines display functional hyper-aggression, the short-attack latency (SAL) mice exhibit violent and pathological forms of aggressive behavior [25]. Furthermore, we have found decreased brain tissue levels of 5-HT in these genetically-selected high-aggressive mice [7], particularly in the violent SAL males [28] and even more prominently after several agonistic encounters with a docile opponent [41]. These findings are in line with the 5-HT deficiency hypothesis related to excessive and pathological aggression [7]. Therefore, we expect that the violent- and/or high-aggressive mice exhibit a constitutionally lower 5-HT biosynthetic capacity than the docile, non- or low-aggressive individuals. In other words, a genetically driven reduction of TPH functionality is expected to be a 5-HT related biochemical trait, characteristic of high-aggressive phenotypes, in particular of the SAL mice.

The present study principally aimed to test this hypothesis by estimating the *in-vivo* 5-HT synthesis rate biochemically via measuring the accumulation of 5-hydroxytryptophan (5-HTP) following treatment with the central aromatic amino-acid decarboxylase inhibitor 3-hydroxybenzylhydrazine (NSD-1015) in the artificially-selected high-and low-aggressive mouse lines.

2. Materials and methods

2.1. Animals

Naïve male mice aged 3–4 months from three genetic selection lines (Groningen: SAL, LAL; Turku: TA, TNA; NC: NC900, NC100) were used as experimental subjects. Short Attack Latency (SAL) and Long Attack Latency (LAL) are outbred strains selected artificially from a wild population in Groningen, The Netherlands [40]. Turku aggressive (TA) and non-aggressive (TNA) are outbred strains obtained through artificial selection from laboratory Swiss albino mice in Turku, Finland [34]. NC900 (aggressive) and NC100 (non-aggressive) are outbred strains selected from laboratory ICR mice in North Carolina [14].

These subjects were kept in groups until weaning (3 weeks after birth), and then the males were housed together with a female of the same line in Makrolon Type II cages (375 cm^3).The litters were culled periodically. The mice were fed *ad-libitum* on standard pellets (AMII, ABDiets, Woerden, The Netherlands) and acidified water. They were exposed to a reversed light-dark cycle of 12 h shifting to darkness at 10:00 h. Each cage was provided with sawdust bedding, shredded paper (Envirodry, The Netherlands), nesting and cardboard tubing enrichment materials. Room temperatures were maintained at 22 ± 2 °C. The animal care complied with the Law on Animal Experimentation and was approved by Institutional Animal Care and Use Committee (IACUC), University of Groningen [D4328A].

2.2. Materials

All chemicals including NSD-1015, 5-HT and 5-HTP used for the standard curve were purchased from Sigma Aldrich, The Netherlands. Perchloric acid, Di-sodium hydrogen phosphate (Na_2HPO_4), Citric acid, EDTA, L-Heptane Sulphonic acid (HAS) and Methanol (MeOH) were purchased from Roche, The Netherlands.

2.3. Measurement of whole-tissue 5-HT and 5-HTP

Mice ($n = 6$ per group) were either treated with double-distilled water or with the aromatic amino-acid decarboxylase inhibitor NSD-1015 (150 mg/kg) subcutaneously 30 min before they were sacrificed after the onset of the dark phase. Totally, 72 male mice were used for the study. Brains were immediately removed and regions containing pre-frontal cortical areas (2 mm of the frontal pole, just anterior to the beginning of the corpus callosum) and midbrain dorsal and median raphe complex areas were rapidly dissected and snap-frozen in liquid nitrogen and stored at −80 °C. The samples were homogenized in 1 ml 0.1 M perchloric acid for 60 s and centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was removed and stored for 1–2 days at −80 °C in order to avoid 5-HT degradation before analysis using HPLC with electrochemical detection.

2.4. HPLC analysis

Tissue supernatants were assayed for 5-HT and 5-HTP using high performance liquid chromatography (HPLC). One hundred microlitres of supernatant were subsequently injected into Gemini C18 110A column (150 mm × 4.60 mm, 5 u, Bester) connected to a detector (analytical cell: ESA model 5011, 0.34 V). The mobile phase consisted of 62.7 mM Na_2HPO_4, 40.0 mM citric acid, 0.27 mM EDTA, 4.94 mM HSA and 10% MeOH (pH 4.1). Known amounts of 5-HT and 5-HTP were run in parallel for standardization. Monoamine/precursor levels were calculated as nmol g^{-1} of wet tissue.

2.5. Data analysis

Synthesis rate and synthesis rate constant of 5-HT were used for the statistical analysis. Data were analyzed separately for each individual brain region using a two-way ANOVA with 'strain' (3 levels: Groningen, Turku and North Carolina) and 'type' (2 levels: High and Low aggressive) as between-subject factors. *Post-hoc* analysis was carried out for the significant effects using Tukey's test.

3. Results

3.1. Synthesis rate

The *in-vivo* 5-HT biosynthesis rates were determined from the initial rate of accumulation of its precursor 5-HTP after inhibition of the central amino-acid decarboxylase. The rates were found to be similar in both the high- and low-aggressive naïve subjects regardless of the brain regions considered (Fig. 1). Univariate ANOVA failed to reveal a 'type'

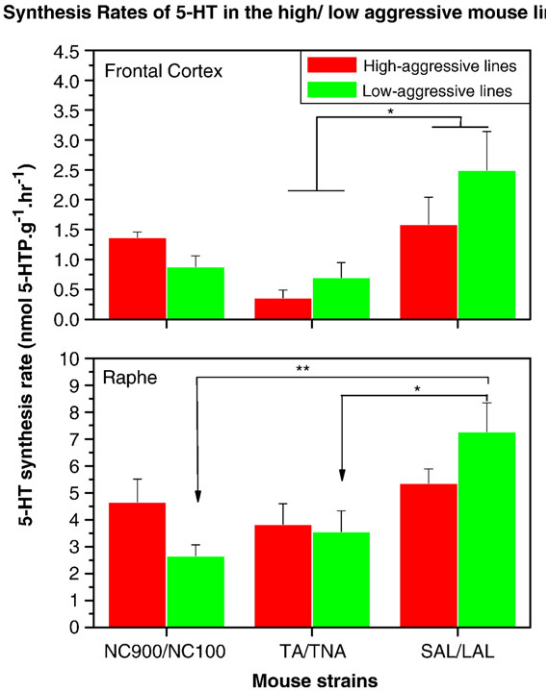

Fig. 1. Shows the synthesis rates of 5-HT in the pre-frontal cortex (top-half) and the raphe nucleus (bottom half) of high- and low-aggressive mouse strains. The synthesis rate is represented as mean ± SEM. Significant *p*-values are represented as * ($p<0.05$) and ** ($p<0.01$).

main effect, but a significant 'strain' effect was observed in both the pre-frontal cortex [$F_{(2,29)}=5.28$; $p<0.05$] and the raphe regions [$F_{(2,29)}=7.95$; $p<0.005$]. *Post-hoc* analysis revealed that the Groningen SAL/LAL strain has a higher 5-HT synthesis rate than the Turku TA/TNA strain ($p<0.01$) in the prefrontal cortex. A similar effect was seen in the raphe brain region, in that the Groningen strains produce more 5-HTP than the Turku strains ($p<0.01$) or the NC 900/100 strains ($p<0.005$). Although no significant 'Selection'×'type' interaction effects was found, a clear trend did appear in the raphe brain region [$F_{(2,29)}=3.32$; $p=0.058$]. *Post-hoc* analysis with Tukey's test revealed significant differences within the low-aggressive mouse lines, in that the LAL mice showed the highest [*5-HTP*] levels than the NC100 ($p<0.005$) or the TNA mice ($p<0.05$). Thus, Groningen strains tend to produce more 5-HTP in both the pre-frontal cortex as well as raphe nucleus.

3.2. [5-HT]

The 5-HT levels were measured from untreated naïve subjects and subjected to analysis. The relevance of assessing untreated 5-HT levels is described under *synthesis rate constant* section below. Univariate ANOVA failed to reveal 'type'-specific main effect as well as 'strain'×'type' interaction effects regardless of the brain region investigated. A significant 'strain' effect however was observed at the pre-frontal cortex [$F_{(2,27)}=3.88$; $p<0.05$]. *Post-hoc* analysis with Tukey's test revealed significantly higher 5-HT levels in the NC strains than with the Groningen strains ($p<0.05$). Thus, 5-HT levels failed to correlate negatively with violent/high aggressive phenotypes in naïve mice selected for high aggression (see Table 1).

3.3. Synthesis rate constant

The TPH activity is measured by the synthesis rate constant of 5-HT. It is obtained by dividing the synthesis rate of *5-HTP* (nmol g^{-1} h^{-1}) by the untreated [5-HT] (nmol g^{-1}). The synthesis rate constants for the pre-frontal cortex were log transformed to ensure the homogeneity of the variances. Similar to the 5-HT biosynthesis rate, the synthesis rate constant failed to reveal any significant 'type' main effect or 'strain'×'type' interaction effects (Table 1). Univariate ANOVA however, showed significant main 'strain' effects at both the pre-frontal cortex [$F_{(2,29)}=7.47$; $p<0.001$] and at the raphe nucleus [$F_{(2,29)}=5.42$; $p=0.01$]. Upon *post-hoc* analysis, the Groningen SAL/LAL strains were shown to have a higher synthesis rate constant in the pre-frontal cortex when compared to both the Turku ($p<0.001$) and NC strains ($p<0.05$). *Post-hoc* analysis for the 'strain' effect in the raphe nucleus revealed NC strains show the lowest synthesis rate constant when compared to Groningen strains ($p<0.01$). The Groningen strains were comparable to Turku strains and both showed highest synthesis rate constants than the NC strains at the raphe nucleus. Thus, the Groningen strains showed enhanced *in-vivo* TPH activity at the pre-frontal cortex than the other strains.

Table 1
Shows both the 5-HT (untreated) and synthesis rate constants of 5-HT for all six mouse lines genetically selected for high/ low aggression.

Strain/PFC	[5-HT] (nmol g^{-1})	Synthesis rate constant (h^{-1})
NC900	3.9 ± 0.33	0.51 ± 0.03
NC100	2.89 ± 0.24	0.39 ± 0.08
TA	1.51 ± 0.60	0.19 ± 0.07
TNA	2.27 ± 0.68	0.32 ± 0.12
SAL	2.07 ± 0.31	1.52 ± 0.43
LAL	3.14 ± 0.30	1.93 ± 0.74
Strain/RN	**[5-HT] (nmol g^{-1})**	**Synthesis rate constant (h^{-1})**
NC900	5.26 ± 0.55	1.79 ± 0.33
NC100	4.25 ± 0.54	0.75 ± 0.12
TA	2.77 ± 0.22	1.79 ± 0.36
TNA	2.75 ± 0.27	1.50 ± 0.33
SAL	3.88 ± 0.18	2.12 ± 0.21
LAL	4.89 ± 0.40	2.31 ± 0.34

(PFC — pre-frontal cortex, RN — raphe nucleus). A summary of the 5-HT biosynthetic activity related parameters in the PFC/RN.

4. Discussion

The present study aimed to reveal a possible negative correlation between TPH activity and aggression by assessing the *in-vivo* synthesis rates of 5-HTP and consequently the synthesis rate constant of 5-HT, in three mouse strains genetically selected for high/low aggression. The *in-vivo* TPH activity has been routinely characterized by estimating the accumulation rates of 5-HTP after inhibition of aromatic L-amino acid decarboxylase by NSD-1015 [8]. Our present findings failed to reveal differences in the synthesis rates or the rate constants of 5-HT between the high/low aggressive mouse strains. Given the above findings, it is evident that the artificially selected high- and low-aggressive mouse lines failed to show any inherent genetic differences with respect to their brain TPH activity.

The pre-frontal cortex and the dorsal/median raphe nuclei in particular were specifically considered for the present study for reasons as described below. The prefrontal cortex has been heavily associated in the inhibitory neural circuitry of aggressive behavior as well as in higher-order social, cognitive and emotional processes [26]. Hypo-function, due to damage, lesions and/or low 5-HT levels, of the prefrontal cortex was found to correlate positively with impulsivity and violence in humans [3,11,17,36]. Furthermore, the low 5-HT levels in the high aggression mouse strains were reported in the pre-frontal cortex in the previous studies [7]. Additionally, the dorsal and median raphe nuclei were considered since they provide the 5-HT neuronal projections to the pre-frontal cortex [35,37–39]. The raphe region is also known to be the seat of synthesis of 5-HT by possessing the highest neural density of TPH [30]. Apart from the functional significance, the ubiquity of a likely negative correlation between TPH activity and violence/aggression was assessed across these aggression-specific brain regions, which are also strongly associated with serotonergic neurotransmission. Other brain regions were not considered for this study owing to their relatively less significance when compared to these brain regions with respect to aggression.

A careful inspection of synthesis rate constants reveals a lack of differential 5-HT levels in these artificially selected genetic strains lacking resident-intruder agonistic experience. In the first instance, this observation seems to contradict our previous findings that showed a consistently low brain 5-HT levels/turnover in the high-aggressive mice [7]. Upon closer scrutiny, it was evident that the low 5-HT levels were observed only in those high-aggressive mice that have been tested repeatedly in the resident-intruder paradigm. This strongly indicates that the 5-HT deficiency is a consequence of acquired victorious resident-intruder agonistic experiences rather than a cause of aggression as a behavior *per se*. Recently, Caramaschi and co-workers [6] found this to be the case with these mice strains in their repeated resident-intruder experiments.

Additionally, from the present findings we can also conclude that a low basal TPH activity is not the direct cause behind the genetic selection of the high-aggressive mice with respect to these brain regions analyzed. Repeated aggressive experiences can nevertheless still indirectly regulate a differential TPH activity in the high and low aggressive mouse lines. If this is the case, a low TPH activity is a consequence of aggression experience rather than a cause, like the 5-HT deficiency as described above in the high aggressive mice. This possibility needs further exploration. However, the effect of experiences on TPH activity is not within the scope of this study.

In addition, several other molecular candidates that are known to regulate 5-HT neurotransmission may be the *direct* underlying causes behind the behavioral differences in aggression of these naïve high/low aggressive mouse strains (e.g., serotonin transporter (SERT), monoamine oxidase (MAO), $5\text{-HT}_{1A/1B}$ autoreceptors). A low 5-HT condition may/(not) be necessarily a mandatory condition but this possibility requires further exploration. Although large bodies of

evidence link low levels of serotonin to enhanced aggressive behavior, several gene knock-out studies have reported a lack of the 'low 5-HT requirement' as well [9,10,16,19,29], thus suggestive of investigating beyond 5-HT levels into the 5-HT regulating candidate genes expression and function.

The synthesis rate constants were however observed to be high in the Groningen strains and this can be attributed to their high synthesis rates of 5-HTP compared to the other selection strains especially at the raphe nucleus. The raphe 5-HT levels were found to be lower in the Turku strains, which in turn lead to the comparable synthesis rate constants across both Turku and Groningen strains (Table 1). Interestingly, the SAL mice produced less 5-HTP than the LAL mice at both the pre-frontal cortex and at the raphe nucleus although non-significantly. Any likely difference in TPH functionality upon agonistic interactions is merely hypothetical at this point of time across these genetically selected strains.

It is not clear at this time as to how one can link a high synthesis rate of 5-HT with simultaneously low intracellular 5-HT levels in the Groningen mice. Additionally, it is also not evident as to why there is a high TPH activity selectively in the Groningen strains. One possibility is that these strains have a high basal 5-HT release with/(out) poor basal 5-HT reuptake rates, which might in turn account for the reduced intracellular 5-HT levels. The latter can also be envisaged to be modulated by other regulatory components including the degradative (MAO) enzyme system as far as direct serotonergic factors are concerned.

Additionally, within the high aggression strains, no significant intra-high aggressive type/intra-selection effects were observed either with respects to SAL, TA and NC900 lines. This was assessed to observe if reduced TPH activity is also typical of the SAL mice if not the other high aggressive mouse strains. This is because the SAL mice were shown to be predisposed to violence while the others were functionally hyperaggressive [25].

This study was carried out during the dark phase when the lights were out. Although the 5-HT levels were known to be highest in the middle of the light phase [24], the Tph2 protein expression for instance, was found to peak around 6 h after the onset of the dark phase [22]. Additionally, aggression is known to peak during the dark phase and thus the biochemical characterization of TPH activity was assessed during this phase of the day.

To conclude, we thus found no differences in 5-HT biosynthetic capacity between the high- and low-aggressive selection lines in both their prefrontal cortical and raphe brain regions. Thus, the underlying inherent genetic differences in aggressiveness observed in these artificially selected mouse strains are not due to constitutional functional differences in their TPH activity. Given the recent classification of the Groningen SAL mice strain as an animal model of violence [25], the lack of differential TPH functionality undermines its importance as a potential candidate gene behind the genetic selection or predisposition for violence/high aggression. Identification of other potential serotonergic correlates using a similar functional approach may reveal their inherent role underlying the different aggressive behavioral phenotypes in general.

Acknowledgements

The authors like to thank Dr Tim Fawcett for the statistical support, Ramon Grannemann for the HPLC analysis and Auke Meinema for animal care.

References

[1] Adams CF, Liley NR, Gorzalka BB. PCPA increases aggression in male firemouth cichlids. Pharmacology 1996;53:328–30.
[2] Beaulieu JM, Zhang X, Rodriguiz RM, Sotnikova TD, Cools MJ, Wetsel WC, Gainetdinov RR, Caron MG. Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. Proc Natl Acad Sci U S A 2008;105:1333–8.
[3] Best M, Williams JM, Coccaro EF. Evidence for a dysfunctional prefrontal circuit in patients with an impulsive aggressive disorder. Proc Natl Acad Sci U S A 2002;99: 8448–53.
[4] Brown GL, Goodwin FK, Ballenger JC, Goyer PF, Major LF. Aggression in humans correlates with cerebrospinal fluid amine metabolites. Psychiatry Res 1979;1:131–9.
[5] Buchanan CP, Shrier EM, Hill WL. Time-dependent effects of PCPA on social aggression in chicks. Pharmacol Biochem Behav 1994;49:483–8.
[6] Caramaschi D, de Boer SF, de VH, Koolhaas JM. Development of violence in mice through repeated victory along with changes in prefrontal cortex neurochemistry. Behav Brain Res 2008;189:263–72.
[7] Caramaschi D, de Boer SF, Koolhaas JM. Differential role of the 5-HT1A receptor in aggressive and non-aggressive mice: an across-strain comparison. Physiol Behav 2007;90:590–601.
[8] Carlsson A, Lindqvist M. Accumulation of 5-hydroxytryptophan in mouse brain after decarboxylase inhibition. J Pharm Pharmacol 1970;22:726–7.
[9] Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Muller U, Aguet M, Babinet C, Shih JC. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science 1995;268:1763–6.
[10] Chiavegatto S, Dawson VL, Mamounas LA, Koliatsos VE, Dawson TM, Nelson RJ. Brain serotonin dysfunction accounts for aggression in male mice lacking neuronal nitric oxide synthase. Proc Natl Acad Sci U S A 2001;98:1277–81.
[11] Coccaro EF, Siever LJ, Klar HM, Maurer G, Cochrane K, Cooper TB, Mohs RC, Davis KL. Serotonergic studies in patients with affective and personality disorders. Correlates with suicidal and impulsive aggressive behavior. Arch Gen Psychiatry 1989;46:587–99.
[12] Couppis MH, Kennedy CH, Stanwood GD. Differences in aggressive behavior and in the mesocorticolimbic DA system between A/J and BALB/cJ mice. Synapse 2008;62:715–24.
[13] Feldman RS, Meyers JS, Quenzer LF. Serotonin. In: Meyer JS, Quenzer LF, editors. Principles of neuropsychopharmacology. 1st ed. MA, USA: Sinauer Associates; 1997. p. 345–89.
[14] Gariepy JL, Lewis MH, Cairns RB. Genes, neurobiology, and aggression: time frames and functions of social behaviors in adaptation. In: Stoff DM, Cairns RB, editors. Aggression and Violence: Genetic, Neurobiological, and Biosocial Perspectives. NJ, USA: Lawrence Erkbaum Associates; 1996. p. 41–63.
[15] Gibbons JL, Barr GA, Bridger WH, Leibowitz SF. Manipulations of dietary tryptophan: effects on mouse killing and brain serotonin in the rat. Brain Res 1979;169: 139–53.
[16] Holmes A, Murphy DL, Crawley JN. Reduced aggression in mice lacking the serotonin transporter. Psychopharmacology (Berl) 2002;161:160–7.
[17] Jankowski MP, Sesack SR. Prefrontal cortical projections to the rat dorsal raphe nucleus: ultrastructural features and associations with serotonin and gamma-aminobutyric acid neurons. J Comp Neurol 2004;468:518–29.
[18] Karl T, Lin S, Schwarzer C, Sainsbury A, Couzens M, Wittmann W, Boey D, von HS, Herzog H. Y1 receptors regulate aggressive behavior by modulating serotonin pathways. Proc Natl Acad Sci U S A 2004;101:12742–7.
[19] Kim DK, Tolliver TJ, Huang SJ, Martin BJ, Andrews AM, Wichems C, Holmes A, Lesch KP, Murphy DL. Altered serotonin synthesis, turnover and dynamic regulation in multiple brain regions of mice lacking the serotonin transporter. Neuropharmacology 2005;49:798–810.
[20] Kubala KH, McGinnis MY, Anderson GM, Lumia AR. The effects of an anabolic androgenic steroid and low serotonin on social and non-social behaviors in male rats. Brain Res 2008;1232:21–9.
[21] Kulikov AV, Osipova DV, Naumenko VS, Popova NK. The C1473G polymorphism in the tryptophan hydroxylase-2 gene and intermale aggression in mice. Dokl Biol Sci 2005;402:208–10.
[22] Malek ZS, Pevet P, Raison S. Circadian change in tryptophan hydroxylase protein levels within the rat intergeniculate leaflets and raphe nuclei. Neuroscience 2004;125:749–58.
[23] Manuck SB, Flory JD, Ferrell RE, Dent KM, Mann JJ, Muldoon MF. Aggression and anger-related traits associated with a polymorphism of the tryptophan hydroxylase gene. Biol Psychiatry 1999;45:603–14.
[24] Martin KF. Rhythms in neurotransmitter turnover: focus on the serotonergic system. Pharmacol Ther 1991;51:421–9.
[25] Natarajan D, de Vries H, Saaltink D, de Boer SF, Koolhaas JM. Delineation of violence from functional aggression in mice: an ethological approach. Behav Genet 2009;39:73–90.
[26] Nelson RJ, Trainor BC. Neural mechanisms of aggression. Nat Rev Neurosci 2007;8: 536–46.
[27] New AS, Gelernter J, Yovell Y, Trestman RL, Nielsen DA, Silverman J, Mitropoulou V, Siever LJ. Tryptophan hydroxylase genotype is associated with impulsive-aggression measures: a preliminary study. Am J Med Genet 1998;81:13–7.
[28] Olivier B, Mos J, Tulp M, Schipper J, Den Daas S, van Oortmerssen GA. Serotonergic involvement in aggressive behavior in animals. In: Van Praag HM, Plutchik R, Apter A, editors. Violence and suicidality — perspectives in clinical and psychobiological research. New York: Brunner/Mazel; 1990. p. 79–137.
[29] Osipova DV, Kulikov AV, Popova NK. C1473G polymorphism in mouse tph2 gene is linked to tryptophan hydroxylase-2 activity in the brain, intermale aggression, and depressive-like behavior in the forced swim test. J Neurosci Res 2008.
[30] Patel PD, Pontrello C, Burke S. Robust and tissue-specific expression of TPH2 versus TPH1 in rat raphe and pineal gland. Biol Psychiatry 2004;55:428–33.
[31] Popova NK, Voitenko NN, Kulikov AV, Avgustinovich DF. Evidence for the involvement of central serotonin in mechanism of domestication of silver foxes. Pharmacol Biochem Behav 1991;40:751–6.
[32] Rujescu D, Giegling I, Bondy B, Gietl A, Zill P, Moller HJ. Association of anger-related traits with SNPs in the TPH gene. Mol Psychiatry 2002;7:1023–9.
[33] Sakowski SA, Geddes TJ, Thomas DM, Levi E, Hatfield JS, Kuhn DM. Differential tissue distribution of tryptophan hydroxylase isoforms 1 and 2 as revealed with monospecific antibodies. Brain Res 2006;1085:11–8.

[34] Sandnabba KN. Selective breeding for isolation-induced intermale aggression in mice: associated responses and environmental influences. Behav Genet 1996;26: 477–88.
[35] Sesack SR, Deutch AY, Roth RH, Bunney BS. Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with *Phaseolus vulgaris* leucoagglutinin. J Comp Neurol 1989;290:213–42.
[36] Siever LJ, Buchsbaum MS, New AS, Spiegel-Cohen J, Wei T, Hazlett EA, Sevin E, Nunn M, Mitropoulou V. d,l-fenfluramine response in impulsive personality disorder assessed with [18F]fluorodeoxyglucose positron emission tomography. Neuropsychopharmacology 1999;20:413–23.
[37] Smiley JF, Goldman-Rakic PS. Serotonergic axons in monkey prefrontal cerebral cortex synapse predominantly on interneurons as demonstrated by serial section electron microscopy. J Comp Neurol 1996;367:431–43.
[38] van der Kooy D, Hunt SP, Steinbusch HW, Verhofstad AA. Separate populations of cholecystokinin and 5-hydroxytryptamine-containing neuronal cells in the rat dorsal raphe, and their contribution to the ascending raphe projections. Neurosci Lett 1981;26:25–30.
[39] van Erp AM, Miczek KA. Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. J Neurosci 2000;20:9320–5.
[40] van Oortmerssen GA, Bakker TC. Artificial selection for short and long attack latencies in wild *Mus musculus domesticus*. Behav Genet 1981;11:115–26.
[41] Veenema AH, Neumann ID. Neurobiological mechanisms of aggression and stress coping: a comparative study in mouse and rat selection lines. Brain Behav Evol 2007;70:274–85.
[42] Vergnes M, Depaulis A, Boehrer A. Parachlorophenylalanine-induced serotonin depletion increases offensive but not defensive aggression in male rats. Physiol Behav 1986;36:653–8.
[43] Zhang S, Sokolchik I, Blanco G, Sze JY. *Caenorhabditis elegans* TRPV ion channel regulates 5HT biosynthesis in chemosensory neurons. Development 2004;131: 1629–38.