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Research report

A pharmacological evidence of positive association between mouse intermale aggression and brain serotonin metabolism

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ABSTRACT

The neurotransmitter serotonin (5-HT) is involved in the regulation of mouse intermale aggression. Previously, it was shown that intensity of mouse intermale aggression was positively associated with activity of the key enzyme of 5-HT synthesis – tryptophan hydroxylase 2 (TPH2) in mouse brain. The aim of the present study was to investigate the effect of pharmacological activation or inhibition of 5-HT synthesis in the brain on intermale aggression in two mouse strains differing in the TPH2 activity: C57BL/6J (B6, high TPH2 activity, high aggressiveness) and CC57BR/Mv (BR, low TPH2 activity, low aggressiveness). Administration of 5-HT precursor L-tryptophan (300 mg/kg, i.p.) to BR mice significantly increased the 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels in the midbrain as well as the number of attacks and their duration in the resident-intruder test. And vice versa, administration of TPH2 inhibitor p-chlorophenylalanine (pCPA) (300 mg/kg, i.p., for 3 consecutive days) to B6 mice dramatically reduced the 5-HT and 5-HIAA contents in brain structures and attenuated the frequency and the duration of aggressive attacks. At the same time, L-tryptophan or pCPA did not influence the percentage of aggressive mice and the attack latency reflecting the threshold of aggressive reaction. This result indicated that the intensity of intermale aggression, but not the threshold of aggressive reaction is positively dependent on 5-HT metabolism in mouse brain.

1. Introduction

In spite of numerous data indicating the involvement of brain serotonin (5-HT) in the regulation of aggressive behavior [1,2], the role of the brain 5-HT system in the mechanism of aggression is obscure. There are evidences that excessive aggression in humans [3,4], non-human primates [5,6] and rodents [7] is accompanied with brain 5-HT hypofunction. This led to the establishment of the dogma that the central 5-HT system inhibits aggression [8,9]. However, it is beyond doubt that aggressive behavior exists in multiple forms classified according to basic motivation into offensive, defensive and predatory aggression [10–12]. These forms of aggressive behavior are regulated by different genetic, neurochemical and hormonal mechanisms [13–17].

Investigators of aggressive behavior also distinguish between normal aggressive reaction typical for given species and pathological, non-adaptive aggression that occurs under specific conditions [for review see [18]]. Such conditions include early social isolation of animals [19], training [20,21], or breeding [22] for violent offensive aggression. These models are characterized by abnormally cruel reactions that are often aimed at unusual opponents (for example, juvenile and anaesthetized animals or potential sexual partners), are not prevented by submissive behavior of the victim [18] and are negatively correlated to tonic activity of the brain 5-HT system [20]. On the contrary, natural, adaptive aggressive behavior, fulfilling communicative functions, seems to be positively related to activity of the brain 5-HT system [2,23–25].

The resident-intruder model imitates naturally occurring intermale agonistic behavior leading to hierarchy establishment in social groups. This type of aggressive reaction has a wide species generality and is frequently used in psychopharmacology [26]. The most common variation of this test includes long-term isolation (for one month and longer) of animals in order to assess higher levels of aggressiveness. However, such isolation leads to unnatural social and sensory deprivation, which is accompanied by substantial changes in brain monoamine levels and behavior [27]. The existing data on pharmacological alteration of 5-HT synthesis and isolation-induced aggression in mice are rather inconsistent and indicate both positive [28–31] and negative [32,33] correlation between 5-HT and aggressiveness.

In contrast to isolation-induced offence, aggressive reaction towards intruders observed in resident males that were not socially deprived is considered more natural and represents a genetically defined adaptive reaction aimed at defense of the territory and

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resources [26,34]. However, this type of aggression is much less studied due to the commonly low levels of spontaneous intermale offence. Previously, we have shown that in inbred mouse strains, the genetically defined intensity of natural (spontaneous) intermale aggression is positively associated with activity of tryptophan hydroxylase 2 (TPH2), the key enzyme of 5-HT synthesis [35,36]. A single nucleotide polymorphism C1473G (rs33849125) in mouse TPH2 gene (*Tph2*), resulting in the Pro447Arg substitution in the enzyme molecule, produces about twofold reduction of the enzyme activity in PC12 cell culture [37] and mouse brain [38–40]. An association between 1473G allele and reduced aggression intensity in 10 inbred mouse strains was revealed [35]. The 1473G allele was transferred from CC57BR/Mv strain onto the genome of C57Bl/6J strain homozygous for 1473C allele. The resulting congenic B6-1473G mice homozygous for 1473G allele had decreased TPH2 activity and intensity of intermale aggression in the resident-intruder test compared with congenic B6-1473C mice homozygous for 1473C allele [40]. A question has arisen whether the decrease in aggressiveness observed in mice homozygous for 1473G allele is indeed produced by the lowered brain 5-HT metabolism.

The main aim of the present study was to investigate the effect of pharmacological alterations of the brain 5-HT metabolism on the expression of mouse spontaneous intermale aggression. It was hypothesized that 5-HT precursor L-tryptophan would increase 5-HT metabolism in the brain and aggression in mice homozygous for 1473G allele, while TPH2 irreversible inhibitor p-chlorophenylalanine (pCPA) would decrease 5-HT metabolism and attenuate intermale aggression in mice homozygous for 1473C allele. A possible nonspecific effect of L-tryptophan and pCPA on motor activity was evaluated in the open field test. The effect of L-tryptophan or pCPA treatments of 5-HT metabolism in the brain was controlled with 5-HT and 5-HIAA levels.

2. Materials and methods

2.1. Animals and treatment

Mice of C57Bl/6J (B6) ($n = 67$) and CC57BR/Mv (BR) ($n = 59$) strains were maintained in the Institute of Cytology and Genetics (Novosibirsk, Russia) at least for 40 generations using brother-sister inbreeding and were highly inbred. The BR strain was created from a hybrid progeny between C57BL and BALB strains and therefore is kindred to the B6 strain [41]. These two strains were chosen because of their difference in the C1473G polymorphism, the brain TPH2 activity, and the aggression intensity. BR mice are homozygous for 1473G allele and characterized by low TPH2 activity and aggression intensity, while B6 mice are homozygous for 1473C allele and characterized by high TPH2 activity and aggression intensity. At the same time, BR and B6 mice did not differ in the percentage of aggressive males [35,40]. The animals were weaned at the age of four weeks, separated by sex, and kept in groups of six animals per cage (40 cm × 25 cm × 15 cm) at standard conditions (air temperature, 22 ± 2°C; relative humidity 65%, and natural illumination, complete feed and water ad libitum).

Experiments were performed on males aged 10–14 weeks. Two to three days before testing, mice were isolated to reduce the group effects on the behavior and the brain 5-HT levels. It has been shown that three days of isolation does not influence intermale aggression [42]. It was hypothesized that pCPA treatment would decrease initially high 5-HT metabolism and aggression in C57BL/6 mice, while L-tryptophan treatment would increase initially low 5-HT metabolism and aggression in CC57BR mice. To test this hypothesis 35 males of the B6 strain were treated for three consecutive days with pCPA (300 mg/kg i.p., *Serva Feinbiochemica*, Heidelberg, Germany), while 29 BR males were received a single i.p. injection with L-tryptophan (300 mg/kg, Reakhim, Moscow). These doses and the treatment protocols for L-tryptophan and pCPA were shown to be effective [43,44]. L-Tryptophan was preferred to 5-hydroxytryptophan because the former was converted to 5-HT only in 5-HT neurons, while the latter increased 5-HT level in all cells containing aromatic L-amino acids decarboxylase including both 5-HT and catecholaminergic neurons and could lead to unspecific action of 5-HT. Control animals were injected i.p. with saline (32 B6 and 30 BR). Forty-three B6 (23 pCPA-treated and 20 saline-treated) and 39 BR (19 L-tryptophan-treated and 20 saline treated) were subjected to test for aggression, than randomly selected 19 B6 and 19 BR were subjected to the open field test in order to evaluate possible nonspecific effects of the treatments on locomotion. Other 24 B6 (12 pCPA-treated and 12 saline-treated) and 20 BR (10 L-tryptophan-treated and 10 saline-treated) were decapitated without testing in order to avoid possible influence of the test procedure on the 5-HT metabolism. The behavioral tests or decapitation were carried out between 12:00 a.m. and 14:00 p.m. one hour after saline, L-tryptophan or after the last pCPA administration.

All experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals and their suffering.

2.2. Behavior tests

2.2.1. Intermale aggression

Intermale aggression was assayed in the resident-intruder test as described elsewhere [35,40]. Briefly, a random-bred adult male of albino mouse (intruder) was introduced into the home cage of the tested male (resident). Each intruder was used no more than five times. Duration of trials was limited to 10 min. The resident which did not attack the intruder during this time was considered as nonaggressive. As soon as a fight began, the number and the duration of attacks were registered during 2 min by an observer blind to treatment, whereupon the experiment was stopped. Spontaneous aggression was characterized by three indices: (1) the level of aggressiveness (predisposition to aggression) evaluated by the percentage of animals exhibiting attack in the group, (2) the threshold of aggressive reaction evaluated by the attack latency (s), the attack latency of the males that did not show aggression was considered to be 600 s, and (3) the intensity of aggression of the fighting mice evaluated by the number of attacks toward the intruder and by the accumulating attacking time (s) (AAT) during which the resident attacked the intruder [45]. The fixed time of fighting registration (2 min) was necessary for reducing the error of evaluation of aggression intensity resulting from an individual variation of the latency of the first attack. In order to avoid influence of the aggressiveness level on the aggression intensity only the aggressive animals were taken into consideration when calculating the mean attack numbers and the mean AAT.

2.2.2. Open-field test

Open-field test was carried out 5 min after the test for aggression. A mouse was placed into a clear white cylindrical Plexiglas arena (40 cm in diameter and 25 cm high) illuminated with two halogen lamps (12 W each) 40 cm under the semitransparent floor, and its movements were recorded for 5 min with a digital camera. The distance run (cm) was automatically measured, while number of rearings was calculated by an observer [46].

All behavioral traits were registered and evaluated with EthoStudio software [46].

2.3. Neurochemical assessments

Animals were decapitated, the brains were rapidly removed, the cortex, hippocampus and midbrains were dissected, frozen in liquid nitrogen and kept at −70 °C until 5-HT and 5-HIAA determination. Subsequently, the tissue samples were homogenized in 200 μl of buffer containing 0.4 M $HClO_4$ (Sigma, USA), 0.27 mM EDTA (Amresco, USA) and 100 ng/ml 3,4-dihydroxybenzylamine (Sigma, USA) as the internal standard. The homogenates were centrifuged and filtered through Whatman CF/C fiberglass filters (Whatman Ltd., UK). The levels of 5-HT and 5-HIAA in the supernatants were then analyzed by HPLC on Nucleosil C8 column (3 μm particle size, L × I.D. 100 mm × 4.6 mm; Sigma–Aldrich, USA) with electrochemical detection (500 mV, Coulochem III; ESA, Inc., USA) and flowcell (BASInc, USA) using solvent delivery module LC-20AD (Shimadzu Corporation, Japan). The mobile phase contained potassium phosphate buffer (100 mM, pH 4.5; Sigma, USA), 0.1 mM Na_2EDTA, 1.4 mM 1-octanesulfonic acid sodium salt (Sigma, USA) and methanol (4 volume percent; Vekton Ltd., Russia) with a flow rate of 0.6 ml/min.

Standard solution containing 2 ng of each 5-HT and 5-HIAA was repeatedly assayed throughout the entire procedure. The heights of 5-HT and 5-HIAA peaks were estimated using MultiChrom v.1.5 software (Ampersand Ltd., Russia) and calibrated against corresponding standards. The contents of 5-HT and 5-HIAA were expressed in μg/g tissue. The index of 5-HT metabolism was calculated as the ratio of 5-HIAA/5-HT.

2.4. Statistics

Numerical values are presented as the mean ± SEM. Data on 5-HT, 5-HIAA levels or 5-HIAA/5-HT ratio in different structures dependent on strain or treatment were processed using two-way analysis of variance (ANOVA) for repeated measures (structure as repeated variable) followed by post hoc Fisher LSD test. Number of attacks, AAT in the resident-intruder test, as well as distance run and number of rearings in the open-field test were processed using one-way ANOVA. Percentage of aggressors was compared by chi-square test.

3. Results

3.1. Comparisons between the saline-treated B6 and BR mice

Highly significant effect of strain ($F_{1,20} = 89.6$, $p < 0.001$), brain structure ($F_{2,40} = 34.2$, $p < 0.001$) and strain × structure interaction

($F_{2,40} = 3.7$, $p = 0.033$) on 5-HT level in the saline-treated animals was revealed. In all structures studied the 5-HT level in B6 mice was significantly higher than in BR mice (cortex: 1.37 ± 0.14 μg/g in B6 vs 0.85 ± 0.05 μg/g in BR, $p < 0.01$; hippocampus: 2.07 ± 0.18 μg/g in B6 vs 1.12 ± 0.05 μg/g in BR, $p < 0.001$; midbrain: 2.72 ± 0.13 μg/g in B6 vs 1.55 ± 0.06 μg/g in BR, $p < 0.001$). Significant effect of strain ($F_{1,20} = 37.4$, $p < 0.001$), brain structure ($F_{2,40} = 237.5$, $p < 0.001$) and strain × structure interaction ($F_{2,40} = 7.3$, $p < 0.01$) on 5-HIAA concentration in the saline-treated mice was shown. The 5-HIAA level in the hippocampus (0.286 ± 0.015 μg/g in B6 vs 0.171 ± 0.004 μg/g in BR, $p < 0.001$) and in the midbrain (0.368 ± 0.016 μg/g in B6 vs 0.306 ± 0.016 μg/g in BR, $p < 0.001$), but not in the cortex (0.116 ± 0.008 μg/g in B6 vs 0.083 ± 0.03 μg/g in BR, $p > 0.05$) was higher in B6 mice compared with BR mice.

The intensity of intermale aggression evaluated by the number of attacks and the accumulating attaching time was significantly higher in the saline-treated B6 mice than in BR mice (number of attacks: 15.0 ± 2.1 in B6 vs 2.78 ± 0.43 in BR, $F_{1,20} = 22.6$, $p < 0.001$; AAT: 31.0 ± 4.8 s in B6 vs 3.57 ± 0.87 in BR, $F_{1,20} = 21.9$, $p < 0.001$). At the same time, no difference in the attack latency (317.4 ± 54.8 s in B6 vs 409.7 ± 52.5 s in BR, $F_{1,38} = 1.49$, $p > 0.05$) and the percentage of aggressive animals between the saline-treated B6 (65%) and BR (55%) was revealed ($\chi^2 = 0.42$, $p > 0.05$).

In the open-field test no differences in the distance run ($F_{1,14} = 3.66$, $p > 0.05$) or the number of rearings ($F_{1,14} < 1$) between the saline treated B6 and BR mice was shown.

3.2. Effects of L-tryptophan treatment on the brain 5-HT and 5-HIAA levels and behavior in BR mice

Significant effect of the L-tryptophan treatment ($F_{1,17} = 9.2$, $p < 0.01$) and structure ($F_{2,34} = 90.7$, $p < 0.001$), but not treatment × structure interaction ($F_{2,34} = 2.7$, $p > 0.05$) on 5-HT level in the brain was revealed. L-tryptophan significantly increased the 5-HT concentration in the midbrain ($p < 0.001$), but not in the cortex ($p > 0.05$) or in the hippocampus ($p > 0.05$) (Fig. 1A). Marked effect of L-tryptophan administration ($F_{1,17} = 176.3$, $p < 0.001$), structure ($F_{2,34} = 209.8$, $p < 0.001$) and treatment × structure interaction ($F_{2,34} = 58.6$, $p < 0.001$) on 5-HIAA level was shown. The precursor significantly increased the 5-HIAA level in the midbrain ($p < 0.001$) and in the hippocampus ($p < 0.001$), but not in the cortex ($p > 0.05$) (Fig. 1B). Significant effect of L-tryptophan administration ($F_{1,17} = 225.4$, $p < 0.001$), structure ($F_{2,34} = 404.1$, $p < 0.001$) and treatment × structure interaction ($F_{2,34} = 105.3$, $p < 0.001$) on 5-HIAA/5-HT ratio was shown. The precursor significantly increased the 5-HIAA/5-HT ratio in all structures studied ($p < 0.001$) (Fig. 1C).

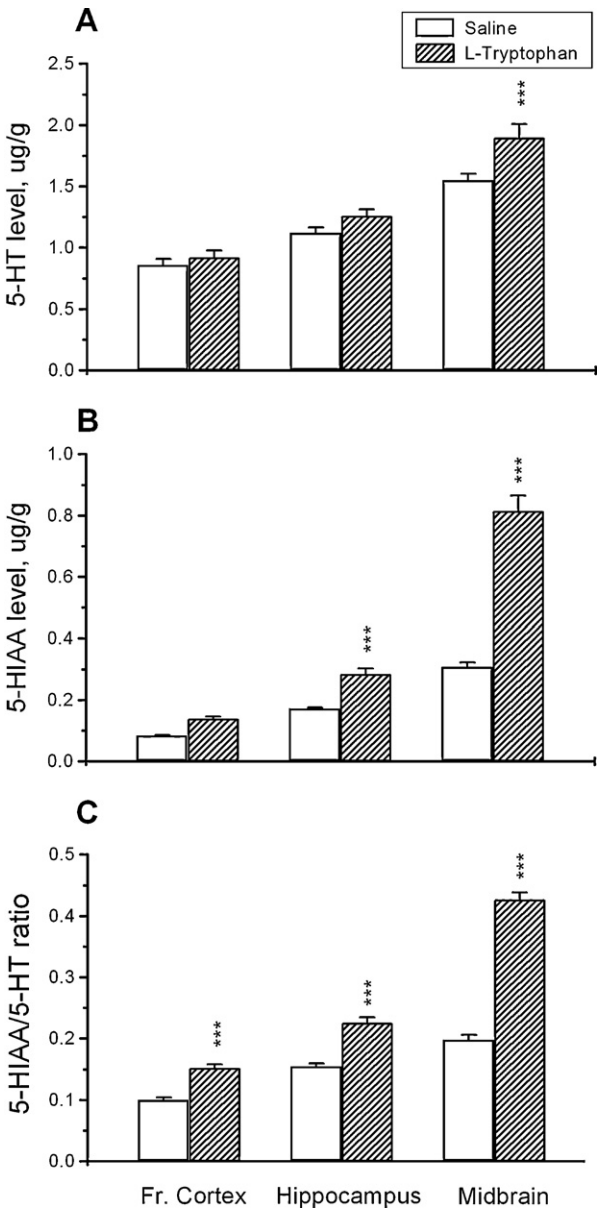

Fig. 1. Levels of 5-HT (A), 5-HIAA (B) and 5-HIAA/5-HT ratio (C) in the cortex, hippocampus and midbrain of CC57BR/Mv mice treated with saline or L-tryptophan (300 mg/kg i.p.). The bars are means ± SEM of 10 animals.
***$p < 0.001$ vs corresponding saline-treated group.

The L-tryptophan administration produced significant increase of the aggression intensity in BR mice. The number of attacks ($F_{1,17} = 12.5$, $p < 0.01$) and AAT ($F_{1,17} = 10.5$, $p < 0.01$) were considerably increased in BR mice treated with L-tryptophan compared with the saline-treated BR mice (Table 1). However, no difference in the attack latency ($F_{1,37} < 1$) and the percentage of aggressive males between the saline- and L-tryptophan-treated BR mice was shown (55% in saline-treated vs 57.9% in tryptophan-treated, $\chi^2 = 0.03$, $p > 0.05$) (Table 1).

Acute treatment with L-tryptophan did not alter the distance run ($F_{1,15} = 1.79$, $p > 0.05$) and the number of rearings ($F_{1,15} = 1.28$, $p > 0.05$) in BR mice in the open field test (Table 1).

3.3. Effects of pCPA treatment on the brain 5-HT and 5-HIAA levels and behavior in B6 mice

Considerable effect of the pCPA treatment ($F_{1,22} = 244.9$, $p < 0.001$), structure ($F_{2,44} = 30.1$, $p < 0.001$), and treatment × structure interaction ($F_{2,44} = 7.8$, $p < 0.001$) on 5-HT level in the brain was revealed. Marked effect of pCPA administration ($F_{1,22} = 425.8$, $p < 0.001$), structure ($F_{2,44} = 166.4$, $p < 0.001$) and treatment × structure interaction ($F_{2,44} = 77.5$, $p < 0.001$) on 5-HIAA level was shown. Significant effect of pCPA administration ($F_{1,18} = 12.8$, $p < 0.002$) and treatment × structure interaction ($F_{2,38} = 7.0$, $p < 0.003$), but not structure ($F_{2,38} = 1.1$, $p > 0.05$) on 5-HIAA/5-HT ratio was demonstrated. The pCPA administration for three successive days produced considerable reduction of the 5-HT ($p < 0.001$) (Fig. 2A) and 5-HIAA ($p < 0.001$) (Fig. 2B) levels in all investigated brain structures of B6 mice. However, the pCPA-induced decrease of the 5-HIAA/5-HT ratio was shown only in the hippocampus ($p < 0.001$) (Fig. 2C).

Treatment with pCPA significantly reduced both the number of attacks ($F_{1,29} = 8.6$, $p < 0.01$) and the AAT ($F_{1,29} = 21.9$, $p < 0.001$) without no effect on the attack latency ($F_{1,41} < 1$) and the percentage of aggressive males (65% in saline-treated vs 78.3% in pCPA-treated mice, $\chi^2 = 0.94$, $p > 0.05$) (Table 2).

Table 1
Effect of L-tryptophan (300 mg/kg) on motor activity in the open field and intermale aggression in CC57BR mice.

Trait	Control	L-tryptophan	P
Open field			
Distance run, cm	1137 ± 130 (n = 8)	1341 ± 85 (n = 9)	$F_{1,15}$ = 1.79, p > 0.05
Number of rearings	20.4 ± 3.3 (n = 8)	27.2 ± 4.9 (n = 9)	$F_{1,15}$ = 1.28, p > 0.05
Intermale aggression			
Number of attacks	2.78 ± 0.43 (n = 9)	10.2 ± 1.95 (n = 10)	$F_{1,17}$ = 12.5, p < 0.003
Accumulating attacking time, s	3.57 ± 0.87 (n = 9)	14.1 ± 3.0 (n = 10)	$F_{1,17}$ = 10.5, p < 0.005
Attack latency, s	409.7 ± 52.5 (n = 20)	376.7 ± 58.3 (n = 19)	$F_{1,37}$ < 1
Percentage of aggressive males	11 of 20 (55%)	11 of 19 (57.9%)	χ^2 = 0.03, p > 0.05

Mice were tested one hour after the saline or L-tryptophan injection.

Treatment with pCPA did not alter the distance run ($F_{1,15}$ < 1) and the number of rearings ($F_{1,15}$ < 1) in B6 mice in the open field test (Table 2).

4. Discussion

The brain 5-HT system is complex and extremely anatomically and functionally expansive. The 5-HT endings are abundant in all brain regions except the cerebellum [47]. Fourteen different 5-HT receptors are coupled with four different signal transduction mechanisms [48,49]. At present, the key role of 5-HT_{1A} and 5-HT_{1B} presynaptic receptors in the spatial and temporal regulation of 5-HT neurotransmission and aggressive behavior is commonly accepted. Agonists of these receptors produce a considerable reduction of rodent offensive aggression [1,50,51]. Moreover, 5-HT_{1B} receptor knockout mice show enhanced aggressive behavior [52–54]. Some investigators interpret these data as an evidence of a negative association between 5-HT neurotransmission and rodent offensive aggression [55,56], while others have quite the reverse opinion [1,50,51]. The latter suggest that 5-HT deficit is associated with impulsivity and results in abnormal forms of aggression, while normal display of offensive aggression is positively related to spike activity of 5-HT neurons [1,50,51].

On contrast to 5-HT_{1A} and 5-HT_{1B} receptors the role of TPH2 in the regulation of aggression remains obscure. TPH2 activity defines the rate of 5-HT synthesis in the brain, since *Tph2* gene knockout [57–59] and irreversible TPH2 inhibitor pCPA [60–63] result in dramatic reduction of 5-HT concentration in the brain. The C1473G polymorphism in *Tph2* gene is the main factor of hereditary variability of the TPH2 activity in the brain of laboratory mice [39]. Earlier we showed the positive interstrain correlation between TPH2 activity and intensity of fighting in inbred mice [35,36]. This result was confirmed with comparison of B6-1473G and B6-1473C mice with respectively 1473G or 1473C alleles transferred to the genome of C57BL/6 strain [40]. B6-1473G mice with lowered TPH2 activity in the brain showed reduced number and duration of attack towards intruder.

It may be hypothesized that C1473G polymorphism in *Tph2* gene alters mouse intermale aggression via modification of 5-HT level and turnover in mouse brain. In the present study a good association between the 5-HT level and turnover in the brain and the aggression intensity in saline-treated (control) B6 and BR mice was demonstrated. In the present study the reduced 5-HT and 5-HIAA levels in the brain structures in the control BR mice compared with the control B6 mice was shown. The aggression intensity in the saline-treated BR was also markedly lower than in the control B6 mice. This result agrees with earlier data [35,36,40,45] and indicates high repeatability and reliability of the model of "spontaneous" aggression used in our experiments. In the present study it was shown that low intensity of aggression in the saline-treated BR mice was accompanied with decreased 5-HT and 5-HIAA levels in their brain structures. This result confirmed the hypothesis on positive association between aggression intensity and TPH2 activity.

Treatment of BR mice with L-tryptophan led to a significant increase of the 5-HT and 5-HIAA levels in the midbrain – the structure containing the bodies of 5-HT neurons. In other structures (cortex and hippocampus) also observed moderate increase of 5-HT and 5-HIAA concentrations. At the same time, the treatment of BR mice with L-tryptophan produced significant increase of 5-HIAA/5-HT ratio in all studied structures. It is worthy to note that in these brain structures of the L-tryptophan-treated BR mice the 5-HT levels were lower, the 5-HIAA levels were near and the 5-HIAA/5-HT ratio were higher compared with the control (saline-treated) B6 mice. So, 300 mg/kg of L-tryptophan was sufficient to significantly increase 5-HT metabolism in the midbrain. Such L-tryptophan-induced enhancement of 5-HT metabolism in the midbrain was accompanied with a substantial rise in the number of attacks and AAT in the resident-intruder test.

Administration of pCPA for three consecutive days to initially highly aggressive B6 mice produced a significant decrease of the 5-HT and 5-HIAA levels in all structures studied. At the same time, the pCPA treatment significantly decreased 5-HIAA/5-HT ratio only in the hippocampus. This moderate decrease of 5-HIAA/5-HT ratio in the pCPA-treated mice seems to result from almost complete reduction of 5-HT synthesis in the brain. The 5-HT, 5-HIAA levels as well as the 5-HIAA/5-HT ratio in the pCPA-treated B6 mice were lower than in the control (saline-treated) BR mice. This dramatic decrease of 5-HT metabolism was accompanied with marked attenuation

Table 2
Effect of pCPA (3 × 300 mg/kg) on motor activity in the open field and intermale aggression in C57BL/6 mice.

Trait	Control	pCPA	P
Open-field			
Distance run, cm	865 ± 57 (n = 8)	847 ± 91 (n = 9)	$F_{1,15}$ < 1
Number of rearings	23.9 ± 2.0 (n = 8)	27.6 ± 5.8 (n = 9)	$F_{1,15}$ < 1
Intermale aggression			
Number of attacks	15.0 ± 2.1 (n = 13)	7.8 ± 1.4 (n = 18)	$F_{1,29}$ = 8.6, p < 0.01
Accumulating attacking time, s	31.0 ± 4.8 (n = 13)	8.6 ± 2.1 (n = 18)	$F_{1,29}$ = 21.9, p < 0.001
Attack latency, s	317.4 ± 54.8 (n = 20)	255.0 ± 42.3 (n = 23)	$F_{1,41}$ < 1
Percentage of aggressive males	13 of 20 (65%)	18 of 23 (78.3%)	χ^2 = 0.94, p > 0.05

Mice were tested one hour after the last saline or pCPA administration.

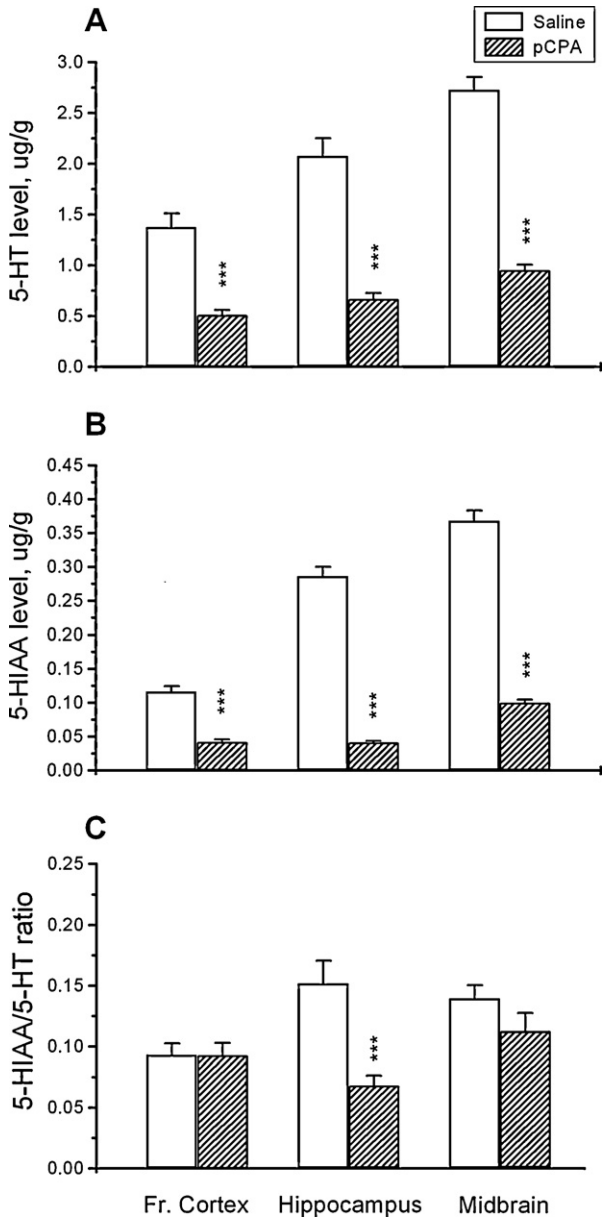

Fig. 2. Levels of 5-HT (A), 5-HIAA (B) and 5-HIAA/5-HT ratio (C) in the cortex, hippocampus and midbrain of C57BL/6J mice treated with saline or pCPA (3 × 300 mg/kg i.p.). The bars are means ± SEM of 12 animals.
***$p < 0.001$ vs corresponding saline-treated group.

of the number of attacks and AAT in the pCPA-treated B6 mice. These data verified the predicted consequences of the hypothesis and indicated a positive association between the brain 5-HT metabolism and the intensity of spontaneous intermale aggression in mice. This result is in accordance with some works showing that adaptive aggressive behavior is positively related to the activity of the brain 5-HT system [1,23–25,50,51]. Moreover, knockout of the gene coding the main enzyme of 5-HT degradation, monoamine oxydase A, significant increased the 5-HT levels in the brain and aggression intensity in mice [64,65]. Therefore, the number of attacks and AAT can be interpreted as indices of normal adaptive aggression aimed to drive intruder out the territory of resident.

These observed alterations in aggressive behavior were not caused by any nonspecific effect of L-tryptophan or pCPA on the motor or exploratory activities of the mice, since the distance run and the number of rearings in the open-field test were not altered with these L-tryptophan or pCPA treatment.

The aggressiveness level did not associated with TPH2 activity in mouse brain [35,36] and it was controlled with different genetic mechanisms than the aggressive intensity [45]. The aggressiveness level seems to reflect the threshold of aggressive reaction, the "hot temper" of animal. Therefore, according to commonly accepted hypothesis on negative association between 5-HT neurotransmission and impulsive aggression [1,50,51,55,56] it could be expected that L-tryptophan would decrease the percentage of aggressive mice or/and increase the attack latency in initially low aggressive BR mice, while pCPA would increase the percentage of aggressive mice or/and decrease the attack latency in initially high aggressive B6 mice. At the present study no association between 5-HT neurotransmission and the aggressiveness level was shown. Although B6 and BR mice differed in 5-HT and 5-HIAA levels in their brain, no difference in the percentage of aggressive males or the attack latency between these strains was shown. Moreover, the percentage of aggressive males or the attack latency were not altered with L-tryptophan or pCPA administration. Therefore, the threshold of resident-intruder aggression is regulated with other 5-HT dependent mechanisms than impulsive aggression.

Numerous observations indicated that 5-HT depletion with pCPA resulted in increase of intermale aggression in rats [66–68]. An inhibitory effect of pCPA on mouse intermale aggression was shown in the present study. The discrepancy between the effects of pCPA on mouse or rat offensive aggression indicates a possible difference in the 5-HT mechanisms of the regulation of this kind of aggression in these species.

5. Conclusions

The results obtained show that: (a) low expression of the aggression intensity was accompanied with genetically defined decrease of the 5-HT metabolism in the brain of BR mice; (b) the enhancement of 5-HT synthesis in the low aggressive BR mice increased both the brain 5-HT metabolism in the midbrain and the intensity of spontaneous intermale aggression; (c) the inhibition of 5-HT synthesis in the highly aggressive B6 strain reduced the brain 5-HT metabolism and suppressed the aggressive behavior; (d) no association between the 5-HT metabolism and the threshold of aggressive behavior in mice was show. Taken together, the data of the present and previous studies [35,40] clearly indicate that intensity of adaptive intermale aggression is positively dependent on brain 5-HT metabolism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2012.04.031.

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