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Veenema, AH; Cremers, Thomas; Jongsma, ME; Steenbergen, PJ; de Boer, Sietse; Koolhaas, JM; Jongsma, Minke E.

Published in:
Psychopharmacology

DOI:
10.1007/s00213-004-2005-5

Publication date:
2005

Psychopharmacology (2005) 178: 151–160
DOI 10.1007/s00213-004-2005-5

ORIGINAL INVESTIGATION

Alexa H. Veenema · Thomas I. F. H. Cremers · Minke E. Jongsma · Peter J. Steenbergen · Sietse F. de Boer · Jaap M. Koolhaas

Differences in the effects of 5-HT_{1A} receptor agonists on forced swimming behavior and brain 5-HT metabolism between low and high aggressive mice

Received: 16 October 2003 / Accepted: 29 July 2004 / Published online: 21 September 2004

Abstract *Rationale:* Male wild house-mice genetically selected for long attack latency (LAL) and short attack latency (SAL) differ in structural and functional properties of postsynaptic serotonergic-1A (5-HT_{1A}) receptors. These mouse lines also show divergent behavioral responses in the forced swimming test (FST, i.e., higher immobility by LAL versus SAL mice). *Objectives:* We investigated whether the line difference in 5-HT_{1A} receptors is associated with a difference in brain 5-HT metabolism, and whether acute administration of a 5-HT_{1A} receptor agonist could differentially affect the behavioral responses of LAL and SAL mice. *Methods:* 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels were measured in homogenates of several brain regions using high-performance liquid chromatography. The behavioral effect of the full 5-HT_{1A} receptor agonist, 8-OH-DPAT, and of the somatodendritic 5-HT_{1A} autoreceptor agonist, S-15535, was examined in the FST. The effect of 8-OH-DPAT on forced swimming-induced 5-HT metabolism in brain homogenates was determined. *Results:* In most brain regions, 5-HT and 5-HIAA levels and 5-HT turnover were not significantly different between LAL and SAL mice. 8-OH-DPAT abolished the behavioral line difference in the FST by reducing immobility in LAL mice and reducing climbing in SAL mice. S-15535 induced a similar behavioral effect to 8-OH-DPAT in SAL mice, but did not alter the behavior of LAL mice. Compared with LAL, forced swimming elicited in SAL mice a higher brain 5-HT turnover, which was potently attenuated by 8-OH-DPAT. *Conclusions:* It is unlikely that the difference in 5-HT_{1A} properties between LAL and SAL mice is an adaptive compensatory reaction to changes in 5-HT metabolism. Although unspecific motor effects, at least in SAL mice, cannot be ruled out, it is suggested that the behavioral effects of 8-OH-DPAT and S-15535 may be mediated by predominant activation of postsynaptic 5-HT_{1A} receptors in LAL mice and by presynaptic 5-HT_{1A} receptors in SAL mice.

Keywords Behavior · Forced swimming test · 8-OH-DPAT · Serotonin (5-HT) · Stress · 5-HT_{1A} receptor · S-15535

A. H. Veenema · S. F. de Boer · J. M. Koolhaas
Department of Animal Physiology, Center for Behavioral and Cognitive Neurosciences, University of Groningen, Groningen, The Netherlands

A. H. Veenema (✉)
Institute for Zoology, University of Regensburg, Universitaetsstr. 31,
93053 Regensburg, Germany
e-mail: alexa.veenema@biologie.uni-regensburg.de
Tel.: +49-941-9433118
Fax: +49-941-9433052

T. I. F. H. Cremers · M. E. Jongsma
Department of Psychiatry, Groningen University Hospital, Groningen, The Netherlands

P. J. Steenbergen
Division of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University Medical Center, Leiden, The Netherlands

Introduction

Dysregulation of central serotonergic (5-HT) neurotransmission, particularly at 5-HT_{1A} receptors, has been implicated in several psychiatric disorders such as anxiety, depression and aggression (Baldwin and Rudge 1995; Maes and Meltzer 1995; Van Praag 2001; Blier and Ward 2003).

In male wild house-mice, genetically selected for low and high aggressive behaviors, a difference in postsynaptic 5-HT_{1A} receptor expression and binding capacity was found. Long attack latency (LAL; low-aggressive to non-aggressive) mice showed lower 5-HT_{1A} receptor expression and binding capacity in hippocampus, frontal cortex and lateral septum than short attack latency (SAL; high-aggressive) mice (Korte et al. 1996). Furthermore, LAL mice showed a lower sensitivity of postsynaptic 5-HT_{1A} receptors (as shown by a lower 8-OH-DPAT-induced hypothermia; Van der Vegt et al. 2001) and an attenuated 5-HT responsiveness in the hippocampus (measured by

means of electrophysiology; Van Riel et al. 2002) than SAL mice. Currently, it is not known, however, whether the observed difference in postsynaptic 5-HT_{1A} receptors is related to a difference in brain 5-HT metabolism between LAL and SAL mice. Downregulation and/or functional desensitisation of 5-HT_{1A} receptors may constitute an adaptive compensatory response to chronically elevated levels of synaptic 5-HT that may be expected to be present in the non-aggressive LAL mice. A previous study by Olivier et al. (1990) showed that the high-aggressive SAL mice had lower whole brain 5-HT levels than the low-aggressive LAL mice, thereby confirming the well-established link between low 5-HT metabolism with high trait-like aggressiveness. The first part of the present study tested the hypothesis that LAL and SAL mice show baseline differences in 5-HT metabolism in several brain regions.

When LAL and SAL mice are subjected to the forced swim test (FST), they show distinctly different behavioral responses. LAL mice display high immobility behavior, whereas SAL mice show high climbing and swimming behaviors (Veenema et al. 2003b). These behavioral responses are representative of the "passive" coping style in LAL mice and the "active" coping style in SAL mice (Benus et al. 1989, 1991a,b; Sluyter et al. 1996). Others have shown that acute treatment with 5-HT_{1A} receptor agonists could effectively decrease immobility behavior in the FST (Wieland and Lucki 1990; Singh and Lucki 1993; Schreiber and De Vry 1993; Detke et al. 1995; O'Neill and Conway 2001). In the second part of this study, we, therefore, tested the effect of two different types of 5-HT_{1A} receptor agonists (the prototypical full 5-HT_{1A} receptor agonist, 8-OH-DPAT, and the preferential somatodendritic 5-HT_{1A} autoreceptor agonist, S-15535) on the behavioral response of LAL and SAL mice in the FST. Finally, in an attempt to explain the behavioral differences in the FST, stress-induced 5-HT metabolism was measured in several brain regions of vehicle-treated mice and 8-OH-DPAT-treated mice.

Materials and methods

Mice

The two mouse lines, genetically selected for LAL and SAL, originated from a colony of wild house mice (Mus musculus domesticus) maintained at the University of Groningen, The Netherlands, since 1971. The mice were housed in plexiglass cages (17×11×13 cm^3) in a room with a 12-h/12-h light/dark cycle (lights on from 0030 hours to 1230 hours). Standard laboratory chow and water were available ad libitum. The mice were weaned at 3–4 weeks of age and were paired male–female at the age of 6–8 weeks. At 14 weeks of age, male mice were tested for their attack latency as described by Van Oortmerssen and Bakker (1981). Briefly, LAL and SAL male mice were confronted with a standard non-aggressive male opponent at the border of their home cage. The time it takes before a LAL or SAL mouse attacks the non-aggressive opponent is measured over three consecutive days. The attack latency score is the mean of these daily scores. Neither LAL nor SAL mice experienced a social defeat. Only non-attacking LAL males and only SAL males with an attack latency of less than 50 s were used for the experiments. The LAL males came from the 36th to the 38th generation of selection and the SAL males from the 62nd to the 63rd generation and were 17 weeks of age (±2 weeks). All experiments were in accordance with the regulations of the Committee for Use of Experimental Animals of the University of Groningen (DEC no. 2326).

Experiment 1: 5-HT_{1A} receptor mRNA expression and binding and brain 5-HT metabolism in LAL and SAL mice

To determine 5-HT_{1A} receptor mRNA expression and binding capacity, LAL and SAL mice (n=8 per line) were decapitated under CO_2 anesthesia during the late light phase (between 0900 hours and 1200 hours). The brains were rapidly removed, quickly frozen in ice-cold p-heptane and stored in −80°C for subsequent in situ hybridisation and autoradiography. To measure brain contents of 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA), LAL and SAL mice were decapitated under CO_2 anesthesia during the late light phase (n=8 per line) and the early dark phase (between 1500 hours and 1800 hours; n=8 per line). The brains were rapidly removed and dissected into nine regions, and stored at −80°C for subsequent measurements of 5-HT and 5-HIAA contents by means of high-performance liquid chromatography (HPLC).

Experiment 2: behavioral effects of 8-OH-DPAT and S-15535 in FST, and the effect of 8-OH-DPAT on forced swimming-induced 5-HT metabolism

Mice received an intraperitoneal (i.p.) injection of saline (vehicle 8-OH-DPAT, n=6 per line), 2 mg/kg 8-OH-DPAT (LAL n=7; SAL n=6), 5 mg/kg 8-OH-DPAT (LAL n=7; SAL n=6), distilled water (vehicle S-15535, n=9 per line), 5 mg/kg S-15535 (LAL n=6; SAL n=8) or 20 mg/kg S-15535 (LAL n=6; SAL n=8) 1 h before they were subjected to the FST. To exclude the possibility that the behavioral effects of the 5-HT_{1A} receptor agonists were due to drug-induced changes in HPA reactivity, mice were decapitated under CO_2 anesthesia 15 min after the FST, and trunk blood was collected to measure plasma corticosterone concentrations. The brains of the 8-OH-DPAT (5 mg/kg) group and the vehicle group were rapidly removed, dissected into nine regions and stored at −80°C for subsequent 5-HT and 5-HIAA content measurements using HPLC.

Drugs

S-15535-3 methanesulfonate [4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine, lot no. E1798, molecular weight 432.5, provided by Institut de Recherches Internationales Servier, France] was dissolved in distilled water. (±) 8-OH-DPAT [8-hydroxy-2-(di-*n*-dipropylamino)tetralin, molecular weight 328, Research Biochemicals International, Natick, MA] was dissolved in sterile saline (0.9% NaCl). The injections were given i.p. in a volume of 0.2 ml/20 g body weight.

Forced swimming test

The present procedure was a modified version of the test described by Porsolt et al. (1977a). Briefly, mice were given a single trial in which they were forced to swim inside a narrow plexiglass cylinder (diameter of 10 cm) in soiled water for 5 min. The depth of the water was 8.5 cm, which enabled the mice to reach the bottom with their tail. The temperature of the water was 25°C. The following three behaviors were recorded using The Observer, version 3.0 (Noldus Information Technology, Wageningen, The Netherlands): (1) immobility (floating in the water without struggling, making only those movements necessary to keep the head above the water); (2) swimming (making active swimming motions and moving around in the cylinder); (3) climbing (making active movements with the forepaws in and out the water, usually directed against the wall). The behavioral scoring was done by a researcher blinded to the treatment condition. Experiments were performed during the late light phase between 1000 hours and 1200 hours.

High-performance liquid chromatography

For determination of 5-HT and 5-HIAA contents, the brains were dissected on a chilled plate. Each brain was divided into nine regions (frontal cortex, septum, striatum, parietal and occipital cortex, hippocampus, amygdaloid region, hypothalamus, cerebellum, and brain stem). Brain sections were put into eppendorf tubes, frozen in nitric oxide and stored at −80°C until the time of assay. Brain monoamine levels were determined using HPLC method with electrochemical detection. Samples were weighed and then homogenized in 1 ml 0.1 M perchloric acid and centrifuged at 17,089 *g* for 10 min at 4°C. The supernatant was removed and assayed for 5-HT and 5-HIAA by injecting 50 µl onto a reversed-phase Supelcosil LC-18-CB column (150×4.6 mm, 3 µm particle size) connected to a detector. The detector was equipped with a glassy carbon electrode set at a potential of 500 mV relative to the Ag/AgCl reference electrode. The mobile phase consisted of 30 mM sodium acetate, 0.8 mM 1-octanesulfonic acid, 0.5 mM EDTA, 12% methanol and 0.8 mM TMA, at pH 4.1, and delivered a flow of 1 ml/min. Known amounts of 5-HT and 5-HIAA (Sigma Chemical, St Louis, USA) were run in parallel throughout the whole procedure for standardization. The detection limit was 0.5 fmol per sample. Monoamine levels were calculated as ng/g tissue. To investigate the specificity of possible line and time differences between LAL and SAL mice, norepinephrine was also measured in the same mice and in the same HPLC run.

In situ hybridization

Brain tissue sections (20 µm) were cut on a cryostat and thaw-mounted on poly-L-lysine coated slides. These slides were stored at −80°C until the time of hybridization. The hybridization protocol was adopted from Meijer et al. (2000). Hybridization was performed using a specific oligonucleotide for the 5-HT_{1A} receptor (45-mer): 5′-TGG-AGA-TGA-GAA-AGC-CAA-TGA-GCC-AAG-TGA-GCG-AGA-TCA-GCG-CAG-3′. To control for specificity of hybridisation, an oligonucleotide was used that was identical except for seven point mutations: 5′-TGT-AGA-TGA-TAA-AGC-AAA-TGA-TCC-AAG-GGA-GCG-CGA-TCA-TCG-CAG-3′. Hybridized slices were exposed to a X-Omat AR film (Kodak, Rochester, NY, USA) for 14 days (5-HT_{1A} receptor). The optical density was determined using an automatic image analysis system (Quantimet 500, Leica, Cambridge). The optical density of the hippocampal CA1 region and of the dorsal raphe nucleus was determined in three and two sections of each mouse, respectively. The values of the sections were averaged per region for each mouse. For tissue background, the optical density of a small area between the CA1 region and the dentate gyrus and a non-hybridized region outside the dorsal raphe nucleus were measured.

Autoradiography

Brain tissue sections (20 µm) were cut on a cryostat and thaw-mounted on gelatine-coated slides. These slides were stored at −80°C until the time of radioligand binding. ^{3}H-8-OH-DPAT binding to brain sections was performed according to Sijbesma et al. (1991), with some minor modifications. Briefly, after 3×10 min preincubation at room temperature, the mounted sections were incubated in 0.17 M Tris–HCl, pH 7.6, containing 4 mM $CaCl_2$, 0.01% ascorbic acid and 10 µM parglyline in the presence of 1.5 nM ^{3}H-8-OH-DPAT [2(*N*,*N*-di[2,3(*n*)-^{3}H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphtalene, s.a. 221 Ci/mmol, Amersham TRK 850] for 60 min at room temperature. Following incubation, the slides were washed at 4°C in incubation buffer (3×90 s), in distilled water (10 s) and dried in a stream of cold air. Non-specific binding was determined in the presence of 1 µM 5-HT. Sections were exposed to ^{3}H-sensitive film (Hyperfilm, Amersham) together with a standard scale (^{3}H-microscales, Amersham) and exposed at room temperature for 8 weeks. An automatic image-analysis system (Quantimet 500, Leica, Cambridge) was used to measure optical

density. The optical density was determined in the lateral septum, frontal cortex, and hippocampus (CA1 region and dentate gyrus) in three sections per mouse and in the dorsal raphe nucleus in two sections per mouse. The values of the sections were averaged per region for each mouse. ^{3}H-8-OH-DPAT binding was calculated in fmol/mg tissue.

Radioimmunoassay for corticosterone

Blood samples were centrifuged at 2,600*g* for 10 min at 4°C. Plasma samples were stored at −20°C until assayed. Plasma corticosterone was determined in duplo using a commercially available radioimmunoassay kit (Mouse Corticosterone RIA Kit, ICN Biomedicals, Costa Mesa, CA, USA). The detection limit of the assay was 3 ng corticosterone/ml with an intra-assay variance of 4.4% and inter-assay variance 6.5%.

Statistical analysis

A Student's *t*-test was used to compare 5-HT$_{1A}$ receptor mRNA expression and binding in several brain regions between LAL and SAL mice. Analysis of variance (ANOVA) was used to determine line, time, and interaction effects of 5-HT, 5-HIAA and 5-HIAA/5-HT ratio in LAL and SAL mice. The behavioral effects of 8-OH-DPAT and S-15535 in the FST were determined using a multivariate ANOVA. Effects of 8-OH-DPAT and S-15535 on plasma corticosterone, and the effects of 8-OH-DPAT on 5-HT, 5-HIAA and 5-HIAA/5-HT ratio were analyzed using ANOVA. When significance was revealed, appropriate pairwise comparisons (LSD test) were executed based on the estimated marginal means. For all tests, the software package SPSS (version 11) was used and the level of significance was $P<0.05$. Data are presented as mean ± SEM.

Results

Experiment 1: 5-HT$_{1A}$ receptor mRNA expression and binding and brain 5-HT metabolism in LAL and SAL mice

5-HT$_{1A}$ receptor mRNA expression and binding capacity (Fig. 1)

The mRNA expression of the 5-HT$_{1A}$ receptor in the CA1 region of the hippocampus was significantly lower in LAL mice than in SAL mice ($P<0.005$), whereas no difference was observed in the dorsal raphe nucleus (Fig. 1a). Furthermore, a similar line difference was found for hippocampal 5-HT$_{1A}$ receptor binding capacity. ^{3}H-8-OH-DPAT binding in LAL mice was significantly lower in the CA1 region ($P<0.001$) and the dentate gyrus ($P<0.05$) than in SAL mice (Fig. 1b). No line difference in ^{3}H-8-OH-DPAT binding was observed in the lateral septum, frontal cortex and dorsal raphe nucleus (Fig. 1b).

Baseline 5-HT and 5-HIAA concentrations in several brain regions (Fig. 2)

Only a time effect was found for 5-HIAA content in all brain regions except the hypothalamus: frontal cortex ($F_{1,26}=17.437$, $P<0.001$), septum ($F_{1,27}=6.742$, $P<0.05$), striatum ($F_{1,25}=11.091$, $P<0.005$), parietal and occipital cortex ($F_{1,26}=5.016$, $P<0.05$), hippocampus ($F_{1,26}=5.016$, $P<0.05$), amygdaloid region ($F_{1,23}=5.909$, $P<0.05$), cerebellum ($F_{1,23}=5.367$, $P<0.05$) and brain stem ($F_{1,24}=11.557$, $P<0.005$). Here, 5-HIAA content was significantly higher during the late light phase than the early dark phase in the frontal cortex ($P<0.01$, both mouse lines), septum ($P<0.05$, only LAL), striatum ($P<0.01$, only SAL), parietal and occipital cortex ($P<0.05$, only LAL), hippocampus ($P<0.05$, only SAL) and brain stem ($P<0.05$, both mouse lines) [(Fig. 2a)].

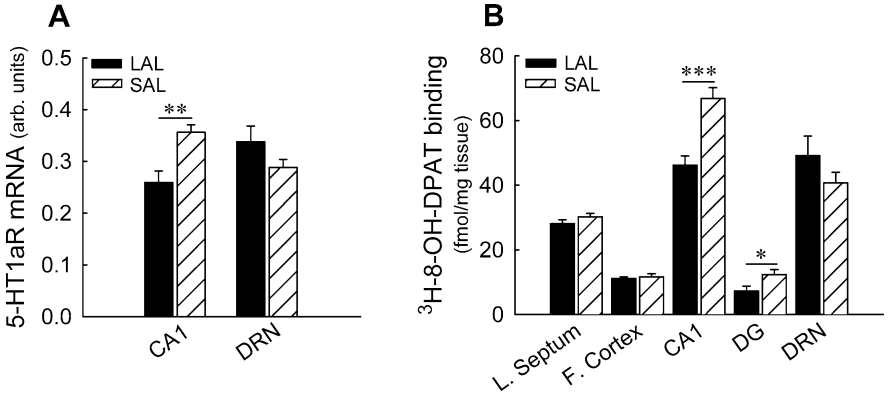

Fig. 1 5-HT$_{1A}$ receptor mRNA expression and binding in several brain regions of long attack latency (LAL) and short attack latency (SAL) mice. **a** LAL mice showed lower 5-HT$_{1A}$ receptor mRNA expression in the CA1 region of the hippocampus than SAL mice, whereas no difference was found in the dorsal raphe nucleus (DRN). **b** ^{3}H-8-OH-DPAT binding was lower in LAL mice in the CA1 region and dentate gyrus (DG) than in SAL mice. No significant line differences were found in the lateral septum, frontal cortex or DRN. *$P<0.05$, **$P<0.005$, ***$P<0.001$, Student's *t*-test

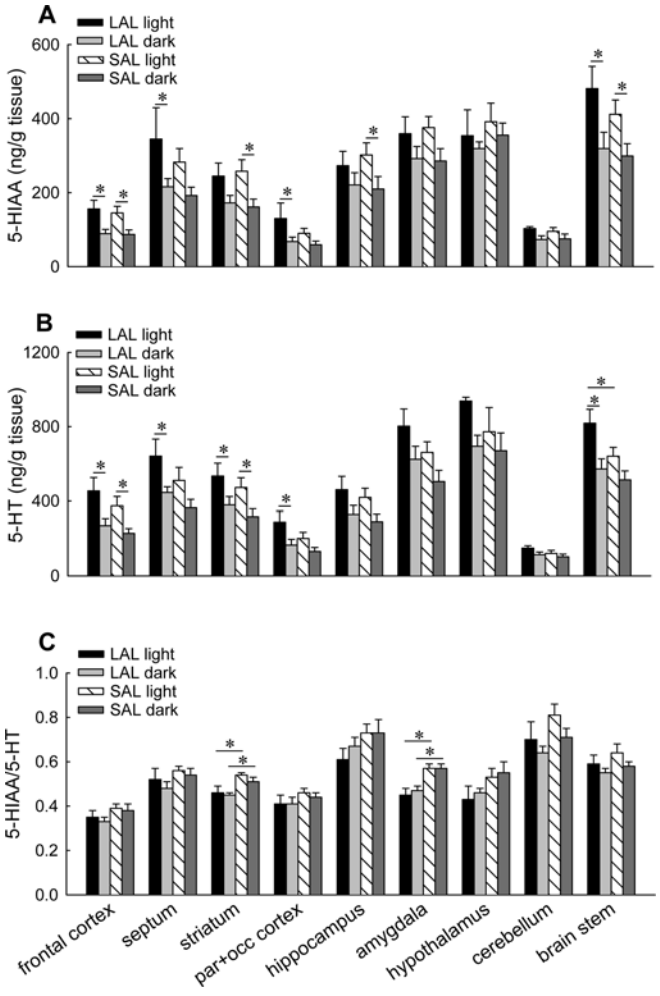

Fig. 2 Serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) contents were measured in the homogenate of several brain regions of long attack latency (LAL) and short attack latency (SAL) mice, sacrificed during the late light phase or early dark phase. 5-HIAA (**a**) and 5-HT (**b**) contents were significantly higher during the late light phase than the early dark phase in several brain regions in both mouse lines. **b** LAL mice showed significantly higher 5-HT contents in the brain stem than SAL mice during the late light phase. **c** 5-HIAA/5-HT ratio was significantly lower in LAL mice than in SAL mice in striatum and amygdaloid region. *P at least <0.05, pairwise comparisons (LSD test) following multivariate ANOVA

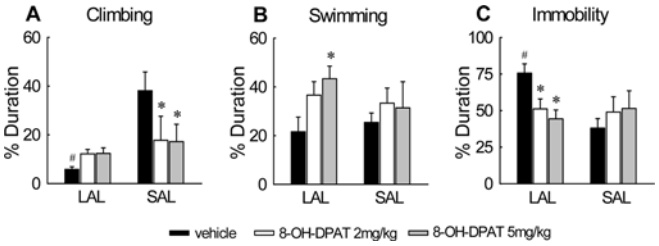

Fig. 3 Effect of a single injection (i.p.) of 8-OH-DPAT (2 mg/kg and 5 mg/kg) on the behavior of long attack latency (LAL) and short attack latency (SAL) mice in the forced swimming test. **a** LAL vehicle mice showed significantly less climbing than SAL vehicle mice. 8-OH-DPAT (2 mg/kg and 5 mg/kg) decreased climbing in SAL mice. **b** 8-OH-DPAT (5 mg/kg) increased swimming in LAL mice. **c** LAL vehicle mice showed significantly higher immobility than SAL vehicle mice. 8-OH-DPAT (2 mg/kg and 5 mg/kg) reduced immobility in LAL mice. *At least P<0.05 versus vehicle, $^{\#}$at least P<0.05 versus SAL, pairwise comparisons (LSD test) following multivariate ANOVA

Only in the brain stem was a line effect observed for 5-HT content ($F_{1,24}$=5.273, P<0.05). Here, LAL mice showed higher 5-HT levels than SAL mice (light phase, P<0.05; Fig. 2b). A time effect was found for 5-HT content in all brain regions except the hypothalamus and cerebellum: frontal cortex ($F_{1,26}$=14.711, P<0.005), septum ($F_{1,27}$=8.834, P<0.01), striatum ($F_{1,25}$=10.487, P<0.005), parietal and occipital cortex ($F_{1,26}$=7.493, P<0.05), hippocampus ($F_{1,26}$=7.344, P<0.05), amygdaloid region ($F_{1,23}$=6.623, P<0.05), brain stem ($F_{1,24}$=13.168, P<0.005). Subsequent pairwise comparisons revealed that 5-HT content was higher during the late light phase than the early dark phase in frontal cortex (P<0.01, both mouse lines), septum (P<0.05, only LAL), striatum (P<0.05, both mouse lines), parietal and occipital cortex (P<0.05, only LAL) and in the brain stem (P<0.005, only LAL) (Fig. 2b).

A line effect was found for 5-HIAA/5-HT ratio in striatum ($F_{1,25}$=18.555, P<0.001), hippocampus ($F_{1,26}$=4.339, P<0.05), amygdaloid region ($F_{1,23}$=33.104, P<0.001), hypothalamus ($F_{1,19}$=6.942, P<0.05) and cerebellum ($F_{1,23}$=5.077, P<0.05). Pairwise comparisons, however, revealed a significantly lower 5-HIAA/5-HT ratio in LAL than SAL mice only in striatum (P<0.005, light; P<0.05, dark) and in the amygdaloid region (P<0.001, light; P<0.005, dark) (Fig. 2c).

To investigate the specificity of the line and time differences in 5-HT and 5-HIAA, norepinephrine was also measured in the same mice. No line or time difference was found for norepinephrine in any of the brain regions measured (data not shown).

Experiment 2: behavioral effects of 8-OH-DPAT and S-15535 in FST, and the effect of 8-OH-DPAT on forced swimming-induced 5-HT metabolism

Effect of 8-OH-DPAT on forced swimming behavior

A line effect was found for climbing ($F_{1,32}$=11.101, P<0.005) and a line×treatment interaction was observed for climbing ($F_{2,32}$=4.358, P<0.05) and immobility ($F_{2,32}$=4.772, P<0.05). Significantly lower climbing (P<0.005; Fig. 3a) and higher immobility (P<0.005; Fig. 3c) behavior was observed in vehicle-treated LAL mice than in vehicle-treated SAL mice. These line differences were abolished by treatment with 8-OH-DPAT. This 5-HT$_{1A}$ receptor agonist induced in LAL mice a significant increase in swimming (P<0.05, 5 mg/kg; Fig. 3b) and a decrease in immobility (P<0.05, 2 mg/kg; P<0.01, 5 mg/kg; Fig. 3c) compared with LAL vehicle. In SAL mice, 8-OH-DPAT induced a significant decrease in climbing (P<0.05, 2 mg/kg; P<0.01, 5 mg/kg; Fig. 3a) compared with SAL vehicle.

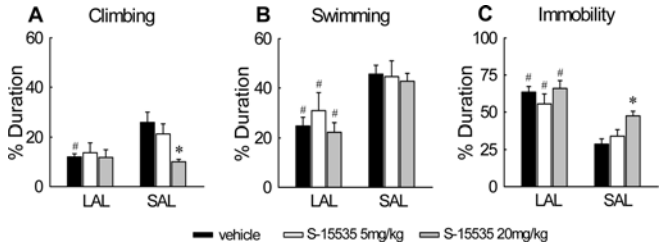

Fig. 4 Effect of a single injection (i.p.) of S-15535 (5 mg/kg and 20 mg/kg) on the behavior of long attack latency (LAL) and short attack latency (SAL) mice in the forced swimming test. **a** LAL vehicle mice showed significantly less climbing than SAL vehicle mice. S-15535 (20 mg/kg) decreased climbing in SAL mice. **b** Swimming was significantly lower in all groups of LAL mice than SAL mice. **c** Immobility was significantly higher in all LAL groups than SAL groups. S-15535 (20 mg/kg) increased immobility in SAL mice. *At least $P<0.05$ versus vehicle, #at least $P<0.05$ versus SAL, pairwise comparisons (LSD test) following multivariate ANOVA

Effect of S-15535 on forced swimming behavior

Line effects were observed for climbing ($F_{1,40}=5.944$, $P<0.05$), swimming ($F_{1,40}=27.511$, $P<0.001$) and immobility ($F_{1,40}=57.976$, $P<0.001$) behavior. Vehicle-treated LAL mice showed less climbing ($P<0.005$; Fig. 4a) and swimming ($P<0.001$; Fig. 4b) and more immobility ($P<0.001$; Fig. 4c) behavior than vehicle-treated SAL mice. LAL mice treated with S-15535 showed less swimming ($P<0.05$, 5 mg/kg; $P<0.005$, 20 mg/kg; Fig. 4b) and more immobility ($P<0.005$, both doses; Fig. 4c) behavior than SAL mice treated with S-15535.

A treatment effect was observed for climbing ($F_{2,40}=3.458$, $P<0.05$) and immobility ($F_{2,40}=5.042$, $P<0.05$) behavior. In SAL mice, S-15535 (20 mg/kg) induced a significant decrease in climbing [$P<0.005$ versus SAL vehicle; $P<0.01$ versus SAL S-15535 (5 mg/kg)] (Fig. 4a) and an increase in immobility [$P<0.005$ versus SAL vehicle; $P<0.05$ versus SAL S-15535 (5 mg/kg)] (Fig. 4c) behavior. No effect of S-15535 (5 mg/kg and 20 mg/kg) was observed on the behavior of LAL mice (Fig. 4a–c).

Effect of 8-OH-DPAT and S-15535 on the release of plasma corticosterone in the FST

No treatment effect was observed for 8-OH-DPAT or S-15535 on plasma corticosterone concentrations. A significant line effect was found in the experiment with S-15535 ($F_{1,39}=8.508$, $P<0.001$). Here, LAL mice showed higher plasma corticosterone concentrations than SAL mice [vehicle-treated mice and mice treated with S-15535 (5 mg/kg), $P<0.05$, Table 1].

Effect of 8-OH-DPAT on 5-HT metabolism in LAL and SAL mice after forced swimming

A line effect for 5-HIAA was found in the hippocampus ($F_{1,21}=9.802$, $P<0.01$) and in the amygdaloid region ($F_{1,21}=8.719$, $P<0.01$). 8-OH-DPAT induced significantly lower 5-HIAA contents in LAL than in SAL mice in both brain regions ($P<0.05$; Fig. 5a). A treatment effect for 5-HIAA was found in the hypothalamus ($F_{1,21}=10.322$, $P<0.005$), where SAL mice treated with 8-OH-DPAT showed lower 5-HIAA contents than SAL vehicle ($P<0.005$; Fig. 5a).

A line effect for 5-HT was found in all brain regions measured: frontal cortex ($F_{1,21}=5.386$, $P<0.05$), septum ($F_{1,21}=7.907$, $P<0.05$), striatum ($F_{1,21}=15.036$, $P<0.005$), parietal and occipital cortex ($F_{1,19}=6.520$, $P<0.05$), hippocampus ($F_{1,20}=15.502$, $P<0.005$), hypothalamus ($F_{1,21}=4.549$, $P<0.05$), amygdaloid region ($F_{1,21}=20.138$, $P<0.005$), cerebellum ($F_{1,20}=7.564$, $P<0.05$) and brain stem ($F_{1,20}=28.074$, $P<0.005$). In general, LAL mice showed higher brain 5-HT contents than SAL mice [vehicle: frontal cortex, striatum, amygdaloid region ($P<0.05$), hippocampus and brain stem ($P<0.005$); 8-OH-DPAT: septum, striatum, cerebellum ($P<0.05$), amygdaloid region ($P<0.005$) and brain stem ($P<0.01$); Fig. 5b]. A treatment effect for 5-HT was found only in brain stem ($F_{1,20}=7.604$, $P<0.05$); here, SAL mice treated with 8-OH-DPAT showed higher 5-HT contents than SAL vehicle ($P<0.05$; Fig. 5b).

A treatment effect for 5-HIAA/5-HT ratio was found in the septum ($F_{1,21}=6.827$, $P<0.05$), hypothalamus ($F_{1,21}=7.460$, $P<0.05$), amygdaloid region ($F_{1,21}=7.708$, $P<0.05$), cerebellum ($F_{1,20}=6.411$, $P<0.05$) and brain stem ($F_{1,20}=10.529$, $P<0.005$). A line×treatment interaction was found in the septum ($F_{1,21}=4.378$, $P<0.05$), parietal and occipital cortex ($F_{1,19}=6.898$, $P<0.05$), hippocampus ($F_{1,20}=4.392$, $P<0.05$) and brain stem ($F_{1,20}=4.738$, $P<0.05$). LAL vehicle showed a lower 5-HIAA/5-HT ratio than SAL vehicle in septum, parietal and occipital cortex and brain stem ($P<0.05$; Fig. 5c). SAL mice treated with 8-OH-DPAT showed a lower 5-HIAA/5-HT ratio than SAL vehicle in all brain regions except the frontal cortex and striatum ($P<0.005$; septum, hypothalamus, brain stem; $P<0.05$ all other regions; Fig. 5c).

Table 1 Effect of a single injection with the 5-HT1A receptor agonist 8-OH-DPAT or S-15535 on plasma corticosterone concentrations (μg/dl) in long attack latency (LAL; n=6–9 per group) and short attack latency (SAL; n=6–8 per group) mice, 15 min after the forced swimming test

	LAL	SAL
8-OH-DPAT		
0 mg/kg	48.9±3.6	36.6±5.8
2 mg/kg	44.5±4.6	52.5±6.9
5 mg/kg	45.4±2.6	44.3±5.4
S-15535		
0 mg/kg	52.1±6.1[a]	39.6±3.0
5 mg/kg	54.0±6.2[b]	38.8±4.6
20 mg/kg	50.1±5.3	45.3±3.1

[a]$P<0.05$ versus SAL S-15535 (0 mg/kg)
[b]$P<0.05$ versus SAL S-15535 (5 mg/kg), pairwise comparisons (LSD) following univariate ANOVA

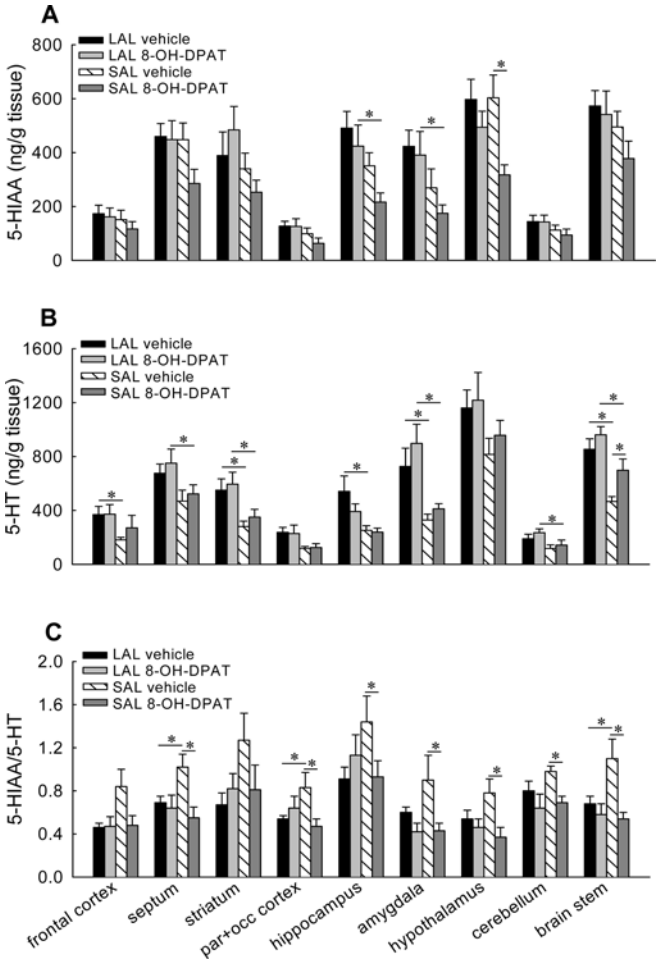

Fig. 5 Effect of 8-OH-DPAT (5 mg/kg) on serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) contents in several brain regions of short attack latency (SAL) and long attack latency (LAL) mice, 15 min after the forced swim test (FST). **a** 8-OH-DPAT induced a decrease in 5-HIAA content in the hippocampus and amygdaloid region in SAL mice relative to that in LAL mice, and in hypothalamus relative to SAL vehicle. **b** 5-HT contents were higher in several brain regions in LAL mice (both groups) than in SAL mice (both groups). 8-OH-DPAT in SAL mice increased 5-HT content in the brain stem compared with vehicle SAL mice. **c** Vehicle LAL mice showed a lower 5-HIAA/5-HT ratio in septum, parietal and occipital cortex and brain stem than vehicle SAL mice. 8-OH-DPAT induced a decrease in 5-HIAA/5-HT ratio in SAL mice in seven of nine brain regions. *At least $P<0.05$, pairwise comparisons (LSD test) following ANOVA

Discussion

In the present study, it was shown that wild house mice selected for LAL and SAL displayed region-specific differences in 5-HT_{1A} receptor expression and binding, and in stress-induced central 5-HT metabolism. These line differences may underlie the distinct behavioral responses to forced swimming as well as the differential behavioral effects of the 5-HT_{1A} receptor ligands 8-OH-DPAT and S-15535 in the FST.

We first confirmed previous findings (Korte et al. 1996; Van Riel et al. 2002) by showing a lower 5-HT_{1A} receptor mRNA expression and binding capacity in the CA1 region and dentate gyrus of the hippocampus in LAL than in SAL mice, and the absence of a line difference in the dorsal raphe nucleus. In contrast with the report by Korte et al. (1996), no line differences were found in two other forebrain regions, the prefrontal cortex and lateral septum. This discrepancy might be due to the difference in time of the day prior to tissue preparation.

Furthermore, a circadian fluctuation was found for 5-HT and 5-HIAA contents (contents were higher during the light than during the dark phase) in both the LAL and SAL mice, which has also been described in other rodent species (Collu et al. 1973; Philo et al. 1977) and seems to be associated with the circadian release of HPA hormones (Aldegunde et al. 1984). Interestingly, in most brain regions, levels of 5-HT, 5-HIAA and 5-HT turnover were not significantly different between LAL and SAL mice. Only in the brain stem was 5-HT content higher, and only in the striatum and amygdaloid region was the 5-HT turnover lower in LAL than SAL mice. Thus, the line differences in 5-HT turnover were found in brain regions other than those in 5-HT_{1A} receptor gene expression and binding. We, therefore, suggest that it is less likely that the observed difference in 5-HT_{1A} properties between SAL and LAL mice is an adaptive compensatory reaction to changes in 5-HT metabolism. These data also signify that we could not confirm that low 5-HT metabolism is linked with high trait aggression as displayed by the SAL mice. These contradictory results suggest a rather complicated pattern of the involvement of 5-HT in low and high trait aggression in these mice. This phenomenon could, however, be specific for these selection lines. Conversely, rats with low and high trait aggression (derived from an unselected strain of wild-type rats) displayed a similar difference in the sensitivity of postsynaptic 5-HT_{1A} receptors as the LAL and SAL mice (Van der Vegt et al. 2001). This suggests that the differences found between LAL and SAL mice are not unique but may rather be a more general characteristic of individuals with low and high trait aggression. Obviously, more delicate techniques such as microdialysis should be applied in future studies to measure extracellular 5-HT in specific brain regions to determine the status of 5-HT neurotransmission in these two mouse lines. Yet, the confirmation of lower hippocampal 5-HT_{1A} receptor gene expression and binding in LAL mice together with lower hippocampal 5-HT responsiveness (Van Riel et al. 2002), lower sensitivity of postsynaptic 5-HT_{1A} receptors (Van der Vegt et al. 2001) and signs of lower 5-HT turnover in striatum and amygdaloid region may indicate a tendency toward lower 5-HT activity in specific brain regions in low-aggressive LAL mice than high-aggressive SAL mice.

In agreement with previous findings (Veenema et al. 2003a,b), forced swimming induced a differential behavioral response in LAL and SAL mice. LAL mice displayed high immobility behavior, whereas SAL mice showed high climbing and/or swimming behavior. This difference in passive versus active behavioral patterns in LAL versus SAL mice is consistent with the idea that these mice differ in the way they cope with environmental

challenges. For example, in the active avoidance test (Benus et al. 1989) as well as in the defensive burying test (Sluyter et al. 1996), LAL mice show freezing behavior, while SAL mice show an active behavioral response by jumping to the other side of the cage (in the active avoidance test) and by burying the shock prod with bedding material (in the defensive burying test).

High immobility behavior in the FST is presumably related to a state of depression-like behavior, as antidepressant drugs are able to decrease immobility behavior in the FST (Porsolt et al. 1977a,b; Porsolt 1979). The high immobility behavior of LAL mice was associated with high 5-HT contents in frontal cortex, striatum, hippocampus, amygdaloid region and brain stem relative to SAL mice. Moreover, 5-HT turnover was lower in all brain regions in LAL than in SAL mice, reaching a significant difference in septum, parietal and occipital cortex, and brain stem. Accordingly, LAL mice showed signs of an attenuated 5-HT responsiveness to forced swimming compared with SAL mice. This study has, however, two limitations. First, the effect of forced swimming on 5-HT responsiveness can only be compared between the two lines, as a control group of mice that did not undergo swimming was not included. Second, it should be considered that these line differences in 5-HT responsiveness could be due to a difference in motor activity in the FST rather than a psychological effect of the FST. In general, it seems that LAL mice are less active than SAL mice. For example, on the elevated plus maze, LAL mice showed a significantly lower number of total entries than SAL mice and, in the open field, LAL mice showed more immobility than SAL mice (Veenema et al. 2003b). However, in the open field, the total distance traveled did not differ between LAL and SAL mice (Veenema et al. 2003b). The latter was explained by the fact that the velocity of movement was significantly higher in LAL than in SAL mice (Veenema et al. 2003b). Accordingly, LAL and SAL mice do not necessarily differ in levels of motor activity. These mice may rather differ in behavioral strategy in which the level of motor activity is determined by their strategy. Nevertheless, it would be of interest to compare the present results with stress-induced 5-HT responsiveness in these mice using other stressors in which physical exercise plays no role (e.g., restraint stress) or is likely to be equal (e.g., rotarod).

Acute administration of the full 5-HT_{1A} receptor agonist, 8-OH-DPAT (2 mg/kg and 5 mg/kg), effectively abolished the line differences in forced swimming behavior. 8-OH-DPAT induced a significant decrease in immobility behavior in LAL mice and a decrease in climbing behavior in SAL mice. The latter finding could be due to a decrease in motor activity typically occurring after administration of 8-OH-DPAT (De Boer et al. 1999). In general, however, 8-OH-DPAT decreases immobility behavior in the FST (Wieland and Lucki 1990; Singh and Lucki 1993; Schreiber and De Vry 1993; Detke et al. 1995; O'Neill and Conway 2001), although, strain differences in the behavioral effects of 8-OH-DPAT have also been reported (Lopez-Rubalcava and Lucki 2000). Moreover, it was found that the antidepressant, fluoxetine, decreased immobility behavior in rats showing "passive" behavior, whereas the same drug increased immobility behavior in rats showing "active" behavior (Taghzouti et al. 1999). Hence, individual differences in passive and active behaviors in the FST determined the effectiveness of drugs such as 8-OH-DPAT and fluoxetine. In the LAL and SAL mice, this may underlie a differential activation or functioning of the 5-HT system, in addition to the differences in 5-HT_{1A} receptors. Indeed, in the present study it was found that the differential behavioral effects of 8-OH-DPAT (5 mg/kg) in the FST were associated with differential changes in 5-HT metabolism between LAL and SAL mice. In LAL mice, 8-OH-DPAT had no effect on brain 5-HT metabolism, whereas 8-OH-DPAT in SAL mice potently attenuated the forced swimming-enhanced 5-HT turnover. Thus, the decrease in climbing behavior induced by 8-OH-DPAT in SAL mice was associated with a decrease in 5-HT metabolism in seven of nine brain regions. The latter could have been mediated by activation of presynaptic 5-HT_{1A} autoreceptors.

To further explore this suggestion, the 5-HT_{1A} receptor agonist S-15535 was used. S-15535 acts as an agonist on the somatodendritic 5-HT_{1A} autoreceptor and has antagonistic properties on postsynaptic 5-HT_{1A} receptors (Millan et al. 1993, 1997; Schreiber et al. 1995). If activation of primarily postsynaptic 5-HT_{1A} receptors induced the behavioral change in LAL mice, it was expected that S-15535 further enhanced immobility behavior or had no effect at all. Indeed, S-15535 (5 mg/kg and 20 mg/kg) did not alter forced swimming behavior in LAL mice. Thus, in LAL mice, the behavioral effects in the FST seem to be mediated primarily by postsynaptic 5-HT_{1A} receptors, an effect which was also shown by others (Lucki et al. 1994). In contrast, S-15535 (20 mg/kg) in SAL mice had a similar behavioral effect as 8-OH-DPAT. This suggests that, in SAL mice, the behavioral effects of S-15535 and of 8-OH-DPAT were mediated by predominant activation of somatodendritic 5-HT_{1A} autoreceptors. These effects of S-15535 or 8-OH-DPAT are unlikely to be attributed to changes in HPA activity as neither ligand affected the release of plasma corticosterone. The observed higher release of plasma corticosterone in LAL than in SAL mice is in agreement with previous findings (Veenema et al. 2003a).

In conclusion, this study demonstrates that genetic selection for trait aggression in male wild house mice is associated with region-specific differences in 5-HT_{1A} receptor properties as well as region-specific differences in swim stress-induced central 5-HT metabolism. These line differences may provide a basis for understanding the distinct behavioral responses to forced swimming, and the differential behavioral effects of the two 5-HT_{1A} receptor ligands in LAL and SAL mice. Further studies are needed to resolve the question whether similar effects of 8-OH-DPAT and S-15535 on behavior and 5-HT responsiveness are found in these mice under conditions in which they are forced to have physical exercise by using, for example, the rotarod. However, even on a rotarod, LAL and SAL mice

may show a difference in motor activity, as, recently, it was shown that rotarod performance was impaired after exposure to chronic stress, and this impairment was not to be due to motor dysfunction (Mizoguchi et al. 2002). Considering the line differences in behavioral coping responses, 5-HT_{1A} receptor properties, 5-HT metabolism and previously found line differences in HPA regulation (Veenema et al. 2003a,b), these two lines of mice represent an interesting animal model to study the genetic basis of individual variation in stress responses.

Acknowledgments The authors would like to thank Gerardus Zuidema and Auke Meinema for excellent animal care. Jan Bruggink and Jan Keijser are appreciated for their technical assistance. A.H. Veenema was supported by the Netherlands Organization for Scientific Research grant NWO-MW 940-70-005.

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