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Enhanced effects of amphetamine but reduced effects of the hallucinogen, 5-MeO-DMT, on locomotor activity in 5-HT_{1A} receptor knockout mice: Implications for schizophrenia

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Abstract

Serotonin-1A (5-HT_{1A}) receptors may play a role in schizophrenia and the effects of certain antipsychotic drugs. However, the mechanism of interaction of 5-HT_{1A} receptors with brain systems involved in schizophrenia, remains unclear. Here we show that 5-HT_{1A} receptor knockout mice display enhanced locomotor hyperactivity to acute treatment with amphetamine, a widely used animal model of hyperdopaminergic mechanisms in psychosis. In contrast, the effect of MK-801 on locomotor activity, modeling NMDA receptor hypoactivity, was unchanged in the knockouts. The effect of the hallucinogen 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) was markedly reduced in 5-HT_{1A} receptor knockout mice. There were no changes in apomorphine-induced disruption of PPI, a model of sensory gating deficits seen in schizophrenia. Similarly, there were no major changes in density of dopamine transporters (DAT) or dopamine D₁ or D₂ receptors which could explain the behavioural changes observed in 5-HT_{1A} receptor knockout mice. These results extend our insight into the possible role of these receptors in aspects of schizophrenia. As also suggested by previous studies using agonist and antagonist drugs, 5-HT_{1A} receptors may play an important role in hallucinations and to modulate dopaminergic activity in the brain.

Keywords

Schizophrenia; 5-HT_{1A} receptors; Serotonin; Dopamine; NMDA receptors; Hallucinogens

1. Introduction

There is extensive evidence for a role of dopaminergic and serotonergic mechanisms in the symptoms of schizophrenia. Most of this knowledge is based on the receptor binding profile and pharmacological mechanism of action of antipsychotic drugs (Millan, 2000; Roth et al., 2004; Seeman, 1987). Recent imaging studies have confirmed the importance of dopamine receptor occupancy for the clinical effects of these drugs and also suggest differences in dopamine release levels in some brain regions between patients with schizophrenia and controls (Laruelle et al., 1999; Stone et al., 2009). While different levels of *in vivo* dopamine

receptor binding are a common feature of all antipsychotic drugs, additional mechanisms may contribute to their effects. Thus, many studies have suggested that combined effects at dopaminergic and serotonergic receptors in the brain may be responsible for some of the differences between various antipsychotics in terms of clinical efficacy and side-effect profile (Meltzer and Huang, 2008; Wood and Wren, 2008; Yadav et al., 2011). In this context, the serotonin-1A (5-HT_{1A}) receptor has received increasing interest (Millan, 2000) as its activation modulates dopaminergic activity and may enhance cognition in schizophrenia (Meltzer and Sumiyoshi, 2008; Sumiyoshi et al., 2008, 2001). For example, micro-dialysis studies have shown that administration of the prototypical 5-HT_{1A} receptor agonist, 8-hydroxy-2-(diethylamino)tetralin (8-OH-DPAT) inhibits dopamine release in the frontal cortex (Kuroki et al., 1996) and nucleus accumbens (Ichikawa and Meltzer, 2000) of rats. However, despite such evidence, the mechanism by which the 5-HT_{1A} receptor is involved in schizophrenia remains unclear.

Therefore, in the present study, we assessed behaviour of 5-HT_{1A} receptor knockout mice in behavioural models of aspects of schizophrenia. These mice have been available for many years but most of the psychopharmacological research with these animals has been directed towards the role of 5-HT_{1A} receptors in anxiety and depression (Parks et al., 1998; Ramboz et al., 1998; Toth, 2003). We used psychotropic drug-induced locomotor hyperactivity as a model of psychosis, with amphetamine-induced increases in dopamine release mimicking subcortical hyperdopaminergia and administration of N-methyl-D-aspartate (NMDA) receptor blockers, such as MK-801 or phencyclidine, used to model hypoglutamatergia (Geyer and Moghaddam, 2002; van den Buuse, 2010). We also tested the effect on locomotor activity of 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT), an indoleamine hallucinogen with non-selective serotonin receptor agonist properties (Halberstadt et al., 2008) on locomotor activity. So far, locomotor hyperactivity studies showed that the effect of MK-801 was not substantially different between 5-HT_{1A} receptor knockout mice and wildtype controls (Castañé et al., 2008; Scorza et al., 2010). The effect of amphetamine or 5-MeO-DMT have not been tested in these animals so far.

Following the results on amphetamine-induced locomotor hyperactivity and the effects of 5-MeO-DMT, we then used another behavioural model with relevance to schizophrenia, prepulse inhibition of acoustic startle (PPI). PPI has been widely used as a model of sensory gating, which is deficient in schizophrenia and some other psychiatric illnesses (Braff et al., 1995). This disruption can be modeled in mice by treatment with psychotropic and dopaminergic drugs, including the dopamine receptor agonist, apomorphine (Geyer et al., 2001; van den Buuse, 2010). There are few previous studies in 5-HT_{1A} receptor knockout mice on nonserotonergic regulation of PPI (Dulawa et al., 2000).

Finally we performed receptor binding autoradiography for dopamine D₁ and D₂ receptors and dopamine transporters (DAT). This was to ascertain whether the knockout mice used here with their constitutive deletion of the 5-HT_{1A} receptor could have developed compensatory mechanisms in central neurotransmitter activity, notably dopamine.

2. Methods

2.1. Animals

The 5-HT_{1A} receptor knockout mice and their wildtype littermate controls were derived from two breeding colonies at the Mental Health Research Institute (MHRI), Melbourne, Australia, and the University of California at San Diego (UCSD), CA, respectively. Breeders on a pure 129Sv background were originally obtained from Dr. Rene Hen, Columbia University, New York, NY (Dulawa et al., 2000). These mice were back-crossed for at least ten generations on a C57Bl/6 background and used to establish a breeding colony

at UCSD (AH, VR). Some of these back-crossed mice were then used to establish another breeding colony at the MHRI in Melbourne, Australia (MvdB, ER, SM). In all cases, heterozygous breeders were used to obtain wildtype and knockout littermates. Heterozygous offspring were not used in the present studies. Genotypes were confirmed at weaning by polymerase chain reaction (PCR) methodology. DNA was extracted from tail samples by the 'HOTSHOT' method and primers were as follows: Primer A (upper primer) 5' AAC TAT CTC ATC GGC TCC TTG 3', Primer B (upper neo primer) 5' GTT AAG AAG GGT GAG AAC AGA 3', Primer C (lower primer) 5' CTT CTT TTC CAC CTT CTT GAC 3' with Primers A and C forming the WT product and Primers B and C forming the KO product.

At the MHRI, the animals were housed in same-sex groups of 1–5 in standard plastic mouse cages with free access to pellet food and tap water. The environment was temperature-controlled (21 ± 2 °C) with a 12 h light/12 h dark schedule (lights on at 7 a.m.). At the time of behavioural testing, the mice were between 12 and 20 weeks of age. All experiments were approved and conducted in accordance to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes set out by the National Health and Medical Research Council of Australia.

At UCSD, mice were housed in groups of four with same-type mice in a vivarium with a 12 L: 12 D schedule (lights off at 0800 h). Food and water were available *ad libitum*. Testing occurred during the dark phase and was in accordance with the "Principles of laboratory animal care" NIH guidelines and with local animal care committee approval.

2.2. Behavioural testing at MHRI: locomotor hyperactivity in photocell cages

Baseline locomotor activity and amphetamine and MK-801-induced locomotor hyperactivity were assessed using the TruScan Photobeam Activity System (Coulbourn Instruments, San Diego, USA). The animals were placed in an enclosure ($25.4 \text{ l} \times 25.4 \text{ w} \times 40.6 \text{ cm h}$) surrounded by a sensor-ring which included a 16×16 array of photobeams. The experiment started with 60 min of baseline locomotor activity measurements and habituation to the test environment. The mice were then briefly taken out of the enclosure and injected and activity was monitored over a subsequent 120 min.

Two cohorts of male and female WT and 5-HT_{1A} receptor knockout mice were used to assess the effect of amphetamine and MK-801, respectively. Each animal was tested three times, once after saline injection, once with a low dose of one of the drugs (1 mg/kg of amphetamine or 0.05 mg/kg of MK-801), and once with the high dose of the same drug (5 mg/kg of amphetamine or 0.25 mg/kg of MK-801) in that animal. The sequence of these treatments was pseudo-randomized between mice so that at the end of the series, each animal had received all doses. There were 8–12 mice per group.

2.3. Behavioural testing at UCSD: behavioural pattern monitor (BPM) photocell cages

Locomotor activity was measured using mouse BPM chambers (San Diego Instruments, San Diego, CA) as previously described (Halberstadt et al., in press, 2009). Each chamber consisted of a clear Plexiglas box with a 30×60 cm hole-board floor enclosed in a ventilated outer box to protect it from light and ambient noise. Infrared photobeams detected the location of the mouse to obtain a measure of locomotor distance moved. The numbers of holepokes and rearings were also recorded but not used in the present experiment. Mice were tested for 60 min in the dark and during the dark phase of their light cycle. During testing, a white noise generator produced background noise at 65 dB.

In the first experiment, WT and KO mice were injected with either vehicle, 5, or 10 mg/kg 5-methoxy-*N,N*-dimethyltryptamine oxalate (5-MeO-DMT; Sigma Chemical Co., St Louis,

MO) in a between-subject design ($n = 6-12/\text{group}$). In the second experiment, WT and KO mice were injected with either vehicle or 1 mg/kg 8-hydroxy-2-(dipropylamino)tetralin hydrobromide (8-OH-DPAT; Sigma Chemical Co.) in a between-subject design ($n = 7-11/\text{group}$). 5-MeO-DMT and 8-OH-DPAT were dissolved in sterile physiological saline and administered 10 min prior to testing. Doses of 5-MeO-DMT refer to the equivalent freebase weight. 5-MeO-DMT and 8-OH-DPAT were administered subcutaneously at a volume of 5 ml/kg bodyweight.

2.4. Behavioural testing at MHRI: PPI

Thirty minutes before each experiment the animals were brought to the testing room to acclimatize to the testing environment. PPI was measured as described previously (Chavez et al., 2009; van den Buuse et al., 2009) using automated startle chambers (SR-Lab; San Diego Instruments, San Diego, USA). Background noise and acoustic pulses were presented through a speaker in the cabinet and responses were measured with the SR-Lab software (San Diego Instruments) running on a computer in an adjacent room. Drugs were injected about 5–15 min before the start of the PPI session. The mice were then given a 3-min acclimation period before the delivery of a total of 104 stimuli. A PPI session included 8 pulse-alone trials at the beginning and end, consisting of a 40 ms burst of 115 dB white noise. The middle 88 trials consisted of the pseudo-random presentation of 16 additional pulse-alone trials, and four groups of 8 prepulse-pulse trials at 2, 4, 8 and 16 dB over baseline. The inter-stimulus interval (ISI) between the prepulse and pulse was set at 30 ms and 100 ms. There were also 8 NOSTIM trials during which no stimulus was delivered.

The same two cohorts of mice used for the locomotor hyperactivity testing were also tested for PPI. There was at least one week between the last locomotor test and the first PPI test.

2.5. Receptor binding autoradiography

Dopamine receptor and transporter binding measurements were done as previously described (Chavez et al., 2009; Choy et al., 2009). Mice were decapitated at 14–16 weeks of age, and brains were frozen on dry-ice and stored at -80°C . Twenty μm sections were cut on a cryostat at -16°C and thaw-mounted onto gelatinised microscope slides. Sections were collected at the level of the nucleus accumbens and caudate putamen. Parallel series of slides were used for total binding and non-specific binding.

For DAT autoradiography, sections were pre-incubated in buffer containing 50 mM NaH_2PO_4 , 70 mM NaCl, 0.025% bovine serum albumin (Sigma), and 1 μM cisflupenthixol (Sigma) for 1 h and then incubated in 2 nM of [^3H]GBR12935 (specific activity 43.0 Ci/mmol; Perkin Elmer) for TB and additional 10 μM GBR12909 (Sigma) for NSB.

For dopamine receptor binding, sections were incubated with the total binding or non-specific binding solutions at room temperature. The total binding solution for dopamine D_2 receptors was applied for 60 min and contained 1 nM of the radioligand, [^3H]YM-09151 (specific activity 85.5 Ci/mmol; Perkin Elmer), supplemented with 0.1 μM pindolol and 0.5 μM DTG (1,3-di-o-tolylguanidine). The non-specific binding solution additionally included the displacer, 10 μM of sulpiride. The total binding solution for dopamine D_1 receptors was applied for 30 min and contained 1 nM of the radioligand, [^3H]SCH23390 (specific activity 73.1 Ci/mmol; Perkin Elmer). The non-specific binding solution additionally included the displacer, 1 μM of cisflupenthixol.

In all protocols, binding incubation was terminated by washes in ice-cold buffer, after which the sections were dried and partially fixed in paraformaldehyde vapour overnight. The slides were then arranged in a Fujifilm BAS 2025 cassette (Berthold, Australia) including

autoradiographic [^3H]micro-scales (Amersham Biosciences). The slides were then apposed to a BAS-TR2025 phosphoimaging plate (Berthold) for 6 days for all studies.

Autoradiographic images were analysed using the Analytical Imaging Station image analysis software (Imaging Research, Ontario, Canada). A standard curve was generated from the micro-scales, allowing the density of the images to be expressed as dpm/mg estimated tissue equivalent (ETE). The specific density was calculated by subtracting total binding minus the non-specific binding levels, and was converted from dpm/mg ETE to fmol/mg ETE using the specific activity and decay factor of the ligand.

2.6. Data analysis and statistics

Data were expressed as the mean $\pm$ standard error of the mean (SEM) and differences between groups were analyzed with analysis of variance (ANOVA) with repeated measures where appropriate (SYSTAT 9.0, SPSS, Chicago, USA). In the locomotor activity experiments at MHRI, between-group statistical factors were Genotype and Sex and within-group, repeated measures factors were Time and Treatment (amphetamine or MK-801 vs. saline). In the locomotor activity experiments at UCSD, between-group statistical factors were Genotype, Sex, and Treatment (5-MeO-DMT or 8-OH-DPAT vs. saline), and Time was a repeated measure (BMDP 8.1, Statistical Solutions, Saugus, MA, USA). In the PPI experiments, between-group factors were Genotype and Sex and the repeated-measures factors were Habituation (four blocks) or Prepulse intensity (four levels at each ISI) and drug Dose. The between-group factor in the binding experiments was Genotype. A p value of $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Amphetamine-induced locomotor hyperactivity

In the first cohort of mice, 5-HT_{1A} knockout mice tended to be slightly less active during the pre-injection habituation period than WT controls (main effect of Genotype: $F(1,36) = 4.8$, $P < 0.05$) but there was no difference between male and female mice (Table 1). Treatment with 1 mg/kg of amphetamine-induced minor hyperlocomotion (main effect of Drug: $F(1,38) = 5.8$, $P < 0.05$) which was not different between 5-HT_{1A} knockout mice and WT controls or between male and female mice (Table 1). Treatment with 5 mg/kg of amphetamine markedly increased locomotor distance moved with a peak at around 20 min after injection (main effect of Drug: $F(1,36) = 285.9$, $P < 0.001$; Drug $\times$ Time interaction: $F(23,828) = 23.7$, $P < 0.001$). This effect was greater in 5-HT_{1A} knockout mice than in WT controls (main effect of Genotype: $F(1,36) = 4.2$, $P < 0.05$; Drug $\times$ Genotype interaction: $F(1,36) = 5.3$, $P < 0.05$) irrespective of the sex of the animals. The genotype difference appeared immediately after the peak response and was maintained for most of the remaining observation period. From 25 to 120 min after injection, the increase in the response to 5 mg/kg of amphetamine was 24.6% in male and 27.5% in female 5-HT_{1A} knockout mice. Amphetamine-induced hyperlocomotion was furthermore greater in female than in male mice (main effect of Sex: $F(1,36) = 18.8$, $P < 0.001$; Drug $\times$ Sex interaction: $F(1,36) = 6.7$, $P < 0.05$) but this was irrespective of the genotype of the animals (Table 1, Fig. 1).

3.2. MK-801-induced locomotor hyperactivity

Similar to the first cohort of mice, baseline locomotor activity during the pre-injection habituation period tended to be lower in the second cohort of 5-HT_{1A} knockouts compared to WT controls ($F(1,35) = 4.0$, $P = 0.052$). Treatment with 0.05 mg/kg of MK-801 had no effect on locomotor activity in any of the groups, although female mice overall had slightly higher locomotor distance moved scores than male mice (main effect of Sex: $F(1,35) = 4.4$, $P < 0.05$; Sex $\times$ Time interaction: $F(23,805) = 1.7$, $P < 0.05$). Treatment with 0.25 mg/kg of

MK-801 significantly increased locomotor distance moved (main effect of Drug: $F(1,35) = 119.3$, $P < 0.001$; Drug $\times$ Time interaction: $F(23,805) = 31.2$, $P < 0.001$) but this effect was similar in 5-HT_{1A} knockouts and WT controls (Table 1, Fig. 1).

3.3. Effect of 5-MeO-DMT in the BPM

There was a main effect of Sex on locomotor activity ($F(1,102) = 8.31$, $P < 0.05$), with female mice displaying slightly higher locomotor activity than male mice. There was, however, no interaction between Sex and Genotype, Sex and Drug, or Sex, Gene, and Drug, so data were collapsed across Sex. Spontaneous locomotor activity in the BPM was initially high but fell to low baseline levels presumably due to habituation to the novel environment (Fig. 2). Analysis of the effect of 5-MeO-DMT revealed main effects of Drug ($F(2,108) = 7.7$, $P < 0.001$) and a Drug $\times$ Genotype $\times$ Time interaction ($F(22,1188) = 10.1$, $P < 0.001$) suggesting time-dependent difference between 5-HT_{1A} knockouts and WT controls. Pair-wise comparisons showed that there was no difference between 5-HT_{1A} knockout mice and WT controls after saline injection. Post-hoc testing also showed that locomotor activity was significantly reduced in WT controls treated with 10 mg/kg of 5-MeO-DMT ($P < 0.05$, Tukey's test) compared to those treated with saline at between 0 and 25 min, although not thereafter. In contrast, there was no effect of 5-MeO-DMT in 5-HT_{1A} knockout mice. In addition, locomotor activity in WT controls treated with 5-MeO-DMT was significantly lower than in similarly-treated 5-HT_{1A} knockout mice between 0 and 20 min (Fig. 2; ($P < 0.05$)). Similar genotype differences were seen with 5 mg/kg of 5-MeO-DMT (data not shown).

Similar to 5-MeO-DMT, treatment with 1 mg/kg of 8-OH-DPAT reduced locomotor activity in WT control mice (Fig. 2). Thus, there was a main effect of Drug ($F(1,72) = 4.7$, $P < 0.05$) and a Drug $\times$ Genotype $\times$ Time interaction ($F(11,792) = 5.9$, $P < 0.001$). Post-hoc testing showed significant reductions of locomotor activity in 8-OH-DPAT-treated WT control mice from 0 to 25 min ($P < 0.05$) but not thereafter. As expected, there was no effect of 8-OH-DPAT in 5-HT_{1A} knockout mice (Fig. 2).

3.4. Apomorphine-induced disruption of PPI and startle habituation

Baseline PPI, measured in saline-treated mice, was not different between 5-HT_{1A} receptor knockout mice and WT controls, either at the 30 ms or 100 ms ISI (Fig. 3). Baseline PPI at the 100 ms ISI was lower in female mice than in male mice (main effect: $F(1,35) = 4.8$, $P < 0.05$) but there was no such difference at the 30 ms ISI. Average startle was also lower in female than in male mice (main effect of Sex: $F(1,35) = 4.9$, $P < 0.05$). There was significant startle habituation over the course of the experiment (main effect of Block: $F(3,105) = 3.9$, $P < 0.05$) which tended to be more pronounced in female mice than in male mice (Block $\times$ Sex interaction: $F(3,105) = 2.7$, $P = 0.051$) but there were no differences between the genotypes in average startle (Fig. 3) or startle habituation (data not shown).

Treatment with apomorphine significantly disrupted PPI at the 30 ms ISI (main effect of Dose: $F(2,70) = 13.8$, $P < 0.001$) with the effect being greater at lower prepulse intensities (data not shown, Dose $\times$ Prepulse Intensity interaction: $F(6,210) = 16.5$, $P < 0.001$). The effect of apomorphine was significant at the 5 mg/kg dose ($F(1,35) = 27.5$, $P < 0.001$), but not the 1 mg/kg dose (Fig. 3). The effect of apomorphine on PPI at the 30 ms ISI was not different between male and female mice or between the genotypes (Fig. 3).

Also at the 100 ms ISI, treatment with apomorphine dose-dependently disrupted PPI (main effect of Dose: $F(2,70) = 26.8$, $P < 0.001$) with the effect being greater at lower prepulse intensities (data not shown, Dose $\times$ Prepulse Intensity interaction: $F(6,210) = 8.5$, $P < 0.001$). The effect of apomorphine was significant at the 5 mg/kg dose ($F(1,35) = 51.6$, $P < 0.001$).

0.001) but only reached trend level at the 1 mg/kg dose ($P=0.083$). PPI at the 100 ms ISI remained significantly higher in apomorphine-treated male mice than in similarly-treated female mice (main effect: $F(1,35)=9.5$, $P<0.05$), however the effect of apomorphine was not different between male and female mice or between WT controls and 5-HT_{1A} receptor knockout mice (Fig. 3).

3.5. Dopamine transporter binding

As expected, [³H]GBR12935 binding was highest in the caudate nucleus, followed by the nucleus accumbens shell and nucleus accumbens core regions with cortical regions being virtually indistinguishable from non-specific (data not shown). The higher level of binding in the caudate nucleus resulted in a main effect of brain region ($F(2,46)=8.5$, $P<0.05$). There was also a marginally significant interaction of brain region $\times$ genotype ($F(2,46)=3.4$, $P<0.05$). If only data from WT mice were analyzed, ANOVA still revealed a significant main effect of brain region ($F(2,26)=12.6$, $P<0.001$) and binding density in the caudate nucleus of male and female WT mice was 27.4 and 37.1% higher than in the nucleus accumbens shell and 21.6 and 17.6% higher than in the nucleus accumbens core, respectively (Table 2). In contrast, analysis of data from 5-HT_{1A} receptor knockout mice showed no difference between the brain regions (Table 2). Analysis of binding in individual brain regions revealed no differences between male and female mice or between WT controls and 5-HT_{1A} receptor knockout mice (Table 2).

3.6. Dopamine D2 receptor binding

Similar to the DAT, very high [³H]YM-09151 binding densities were observed in the caudate nucleus and nucleus accumbens shell and core, but there was no difference between these regions. While there were no main effects of Genotype or Sex, a Genotype $\times$ Sex interaction ($F(1,23)=5.2$, $P<0.05$) reflected differential [³H]YM-09151 binding depending on the sex and on the genotype. Inspection of the data suggested lower binding densities in female WT controls than male WT controls but not different between female and male 5-HT_{1A} receptor knockout mice (Table 3). Indeed, if only data from WT mice were analyzed, a significant main effect of Sex was found ($F(1,11)=19.5$, $P<0.05$) again with no difference between the brain regions analyzed. In contrast, if only data from 5-HT_{1A} receptor knockouts were analyzed, no such sex differences were found. However, if only data from female mice were analyzed, a significant main effect of Genotype could not be demonstrated. Thus, it cannot be concluded that 5-HT_{1A} receptor knockout mice show significantly higher densities of dopamine D₂ receptors.

3.7. Dopamine D₁ receptor binding

Analysis of [³H]SCH23390 binding (Table 4) showed a significant main effect of brain region ($F(2,42)=13.4$, $P<0.001$) and a weak Region $\times$ Genotype $\times$ Sex interaction ($F(2,42)=3.8$, $P<0.05$) but no other main effects or interactions. Analysis of data from female WT mice only revealed a main effect of Region ($F(2,10)=22.9$, $P<0.001$) which was not found in any of the other groups (Table 4). Binding densities in the caudate nucleus in this group were 38.1 and 17.7% higher than in the nucleus accumbens shell and core, respectively. However, for each individual brain region, there were no significant effects of either sex or genotype (Table 4).

4. Discussion

The main findings of this study were that compared to WT littermate controls, 5-HT_{1A} receptor knockout mice showed (1) significantly increased locomotor hyperactivity to treatment with amphetamine but not MK-801; (2) marked attenuation of the effect of the

hallucinogen, 5-MeO-DMT; (3) no changes in PPI or its disruption after treatment with apomorphine; (4) subtle changes in DAT, dopamine D₂ and D₁ receptor binding.

Few previous studies have used 5-HT_{1A} receptor mice in behavioural models with relevance to schizophrenia. At least in rats, amphetamine-induced locomotor hyperactivity is critically dependent on intact dopamine transmission in the nucleus accumbens (French and Vantini, 1984; Kelly and Iversen, 1976). Activation of 5-HT_{1A} receptors in the brain has been shown to inhibit amphetamine-induced dopamine release (Ichikawa et al., 1995) and the enhanced amphetamine-induced locomotor hyperactivity in 5-HT_{1A} receptor knockout mice in the present study could therefore be explained by a constitutive loss of this inhibition. Thus, our results support an inhibitory role of 5-HT_{1A} receptors in the regulation of dopamine release and, potentially, psychosis.

The results of our binding studies show that there were only very subtle changes in the density of DAT and dopamine D₁ and D₂ receptors in 5-HT_{1A} receptor knockout mice compared to controls. Some changes in the differential pattern of density levels between nucleus accumbens sub-regions and caudate nucleus were seen but none of these effects could clearly explain the enhancement of the action of amphetamine on locomotor activity. Therefore, it is possible that the interaction of 5-HT_{1A} receptors with dopamine release is more at the functional level or via interneurons (Fink and Vantini, 2007) rather than via constitutive changes in the expression of the DAT or dopamine receptors.

The effect of 5-HT_{1A} receptor knockout on locomotor hyperactivity did not extend to the effect of MK-801. Previous studies have suggested an important role of nucleus accumbens dopamine release in the effect of NMDA receptor antagonists on locomotor activity (e.g. (French and Vantini, 1984)). If this was the case, a similar enhancement of MK-801-induced locomotor hyperactivity could be expected in the 5-HT_{1A} receptor knockouts as that seen with amphetamine. Such an interaction was not observed, in line with other published findings (Castañé et al., 2008; Scorza et al., 2010). A possible involvement of dopamine release in the behavioural effects of NMDA receptor antagonists has been questioned by several studies (Adams and Moghaddam, 1998; Chartoff et al., 2005) and our results appear to support such a dissociation, at least at the level of 5-HT_{1A} receptor modulation of dopamine release.

While there was a significant interaction of loss of 5-HT_{1A} receptor function with the action of amphetamine on locomotor hyperactivity, there were no changes in the effect of apomorphine on PPI. Our results could indicate that 5-HT_{1A} receptors do not play a major role in dopaminergic regulation of PPI, which would indicate differential regulation of dopaminergic activity involved in locomotor hyperactivity (modeling psychosis) and PPI (modeling sensory gating). However, further experiments need to address the effect of amphetamine on PPI in 5-HT_{1A} receptor knockout mice. In rats it has been shown that the effect of amphetamine on PPI can be blocked by selective lesions of the nucleus accumbens (Swerdlow et al., 1990). It is possible that postsynaptic dopamine receptor activation by apomorphine is normal in 5-HT_{1A} receptor knockouts, whereas amphetamine-induced disruption of PPI could be enhanced, similar to the effect seen in our locomotor hyperactivity studies.

In marked contrast to enhanced locomotor hyperactivity induced by amphetamine, locomotor hypoactivity induced by 5-MeO-DMT, was virtually absent in 5-HT_{1A} receptor knockout mice, indicating an obligatory involvement of these receptors in the effect of this hallucinogen, at least on locomotor activity. The receptor binding profile of hallucinogens, such as 5-MeO-DMT, is that of non-selective 5-HT receptor agonists, with affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors (McKenna et al., 1990; Pierce and Peroutka, 1989).

Previous studies have emphasized the importance of 5-HT_{2A} receptors in the action of several hallucinogens (for references, see (Halberstadt and Nichols, 2010; Nichols, 2004)). However, recently we have shown that the locomotor effect of 5-MeO-DMT could be significantly attenuated by the 5-HT_{1A} receptor antagonist, WAY100, 635 (Halberstadt et al., in press). The present results in 5-HT_{1A} receptor knockout mice confirm these results and suggest an important role of these receptors in the effects of indoleamine hallucinogens, and possibly in schizophrenia (Quednow et al., 2010).

In most of our studies, we were able to compare male and female mice for the effect of deletion of the 5-HT_{1A} receptor. Previously, it has been shown that estrogen modulates dopaminergic activity (Di Paolo, 1994). For example, in rats, we observed that ovariectomy reduced DAT levels in the nucleus accumbens and that estrogen reversed this effect (Chavez et al., 2010). Similarly, ovariectomy increased whereas estrogen treatment reduced dopamine D₂ receptor levels in the nucleus accumbens (Chavez et al., 2010). These effects were associated with functional inhibition of the effect of both apomorphine and 8-OH-DPAT on PPI (Gogos et al., 2010). These results could then predict prominent differences between male and female 5-HT_{1A} receptor knockout mice. However, while the effects of amphetamine and MK-801 on locomotor activity were greater in female mice than in male mice, there were no differences between the sexes in the extent of modulation of the effect of amphetamine or 5-MeO-DMT by the loss of 5-HT_{1A} receptors.

In conclusion, the present studies in 5-HT_{1A} receptor knockout mice extend our insight into the possible role of these receptors in aspects of schizophrenia (Meltzer and Sumiyoshi, 2008; Millan, 2000). As also suggested by previous studies using agonist and antagonist drugs, 5-HT_{1A} receptors may play an important role in the effects of certain hallucinogens and in dopaminergic hyperactivity, but less so in the regulation of PPI, the effects of NMDA receptor hypoactivity or DAT and dopamine receptor binding, at least in mice. Future studies could extend these findings to the action of antipsychotic drugs with mixed dopamine/serotonin receptor binding properties (Van den Buuse and Gogos, 2007).

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References

- Adams B, Moghaddam B. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci*. 1998; 18:5545–5554. [PubMed: 9651235]
- Braff DL, Swerdlow NR, Geyer MA. Gating and habituation deficits in the schizophrenia disorders. *Clin Neurosci*. 1995; 3:131–139. [PubMed: 7583619]
- Castañé A, Artigas F, Bortolozzi A. The absence of 5-HT_{1A} receptors has minor effects on dopamine but not serotonin release evoked by MK-801 in mice prefrontal cortex. *Psychopharmacology*. 2008; 200:281–290. [PubMed: 18594799]
- Chartoff EH, Heusner CL, Palmiter RD. Dopamine is not required for the hyperlocomotor response to NMDA receptor antagonists. *Neuropsychopharmacology*. 2005; 30:1324–1333. [PubMed: 15688082]
- Chavez C, Gogos A, Jones ME, van den Buuse M. Psychotropic drug-induced locomotor hyperactivity and prepulse inhibition regulation in male and female aromatase knockout (ArKO) mice: role of dopamine D₁ and D₂ receptors and dopamine transporters. *Psychopharmacology*. 2009; 206:267–279. [PubMed: 19597801]

- Chavez C, Hollaus M, Scarr E, Pavey G, Gogos A, van den Buuse M. The effect of estrogen on dopamine and serotonin receptor and transporter levels in the brain: autoradiography study. *Brain Res.* 2010; 1321:51–59. [PubMed: 20079719]
- Choy KHC, de Visser Y, Van den Buuse M. The effect of 'two hit' neonatal and young-adult stress on dopaminergic modulation of prepulse inhibition and dopamine receptor density. *Br J Pharmacol.* 2009; 156:388–396. [PubMed: 19154431]
- Di Paolo T. Modulation of brain dopamine transmission by sex steroids. *Rev Neurosci.* 1994; 5:27–42. [PubMed: 8019704]
- Dulawa SC, Gross C, Stark KL, Hen R, Geyer MA. Knockout mice reveal opposite roles for serotonin 1A and 1B receptors in prepulse inhibition. *Neuropsychopharmacology.* 2000; 22:650–659. [PubMed: 10788764]
- Fink KB, Vantini G. 5-HT receptor regulation of neurotransmitter release. *Pharmacol Rev.* 2007; 59:360–417. [PubMed: 18160701]
- French ED, Vantini G. Phencyclidine-induced locomotor activity in the rat is blocked by 6-hydroxydopamine lesion of the nucleus accumbens: comparisons to other psychomotor stimulants. *Psychopharmacology.* 1984; 82:83–88. [PubMed: 6141586]
- Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology.* 2001; 156:117–154. [PubMed: 11549216]
- Geyer, MA.; Moghaddam, B. Animal models relevant to schizophrenia disorders. In: Davis, KL.; Charney, DS.; Coyle, JT.; Nemeroff, CB., editors. *Neuropsychopharmacology - The Fifth Generation of Progress.* Lippincott Williams & Wilkins; Philadelphia: 2002. p. 689-701.
- Gogos A, Kwek P, Chavez CA, van den Buuse M. Estrogen treatment blocks 8-OH-DPAT- and apomorphine-induced disruptions of prepulse inhibition: involvement of dopamine D₁ or D₂, serotonin 5-HT_{1A}, 5-HT_{2A} or 5-HT₇ receptors. *J Pharmacol Exp Ther.* 2010; 333:218–227. [PubMed: 20042529]
- Halberstadt AL, Buell MR, Masten VL, Risbrough VB, Geyer MA. Modification of the effects of 5-methoxy-N, N-dimethyltryptamine on exploratory behavior in rats by monoamine oxidase inhibitors. *Psychopharmacology.* 2008; 201:55–66. [PubMed: 18604652]
- Halberstadt AL, Koedood L, Powell SB, Geyer MA. Differential contribution of serotonin receptors to the behavioral effects of indolamine hallucinogens in mice. *J Psychopharmacol.* in press. 10.1177/0269881110388326
- Halberstadt, AL.; Nichols, DE. Serotonin and serotonin receptors in hallucinogen action. In: Muller, C.; Jacobs, B., editors. *Handbook of the Behavioral Neurobiology of Serotonin.* Academic Press; London: 2010. p. 621-636.
- Halberstadt AL, van der Heijden I, Ruderman MA, Risbrough VB, Gingrich JA, Geyer MA, Powell SB. 5-HT_{2A} and 5-HT_{2C} receptors exert opposing effects on locomotor activity in mice. *Neuropsychopharmacology.* 2009; 34:1958–1967. [PubMed: 19322172]
- Ichikawa J, Kuroki T, Kitchen MT, Meltzer HY. R(+)-8-OH-DPAT, a serotonin 1A receptor agonist, inhibits amphetamine-induced dopamine release in rat striatum and nucleus accumbens. *Eur J Pharmacol.* 1995; 287:179–184. [PubMed: 8749033]
- Ichikawa J, Meltzer HY. The effect of serotonin 1A receptor agonism on antipsychotic drug-induced dopamine release in rat striatum and nucleus accumbens. *Brain Res.* 2000; 858:252–263. [PubMed: 10708676]
- Kelly PH, Iversen SD. Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. *Eur J Pharmacol.* 1976; 40:45–56. [PubMed: 1033072]
- Kuroki T, Ichikawa J, Dai J, Meltzer HY. R(+)-8-OH-DPAT, a 5-HT_{1A} receptor agonist, inhibits amphetamine-induced serotonin and dopamine release in rat medial prefrontal cortex. *Brain Res.* 1996; 743:357–361. [PubMed: 9017269]
- Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R. Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol Psychiatry.* 1999; 46:56–72. [PubMed: 10394474]

- McKenna DJ, Repke DB, Lo L, Peroutka SJ. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. *Neuropharmacology*. 1990; 29:193–198. [PubMed: 2139186]
- Meltzer HY, Huang M. In vivo actions of atypical antipsychotic drug on serotonergic and dopaminergic systems. *Prog Brain Res*. 2008; 172:177–197. [PubMed: 18772033]
- Meltzer HY, Sumiyoshi T. Does stimulation of 5-HT_{1A} receptors improve cognition in schizophrenia? *Behav Brain Res*. 2008; 195:98–102. [PubMed: 18707769]
- Millan MJ. Improving the treatment of schizophrenia: focus on serotonin (5-HT)_{1A} receptors. *J Pharmacol Exp Ther*. 2000; 295:853–861. [PubMed: 11082417]
- Nichols DE. Hallucinogens. *Pharmacol Ther*. 2004; 101:131–181. [PubMed: 14761703]
- Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. Increased anxiety of mice lacking the serotonin 1A receptor. *Proc Natl Acad Sci USA*. 1998; 95:10734–10739. [PubMed: 9724773]
- Pierce PA, Peroutka SJ. Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. *Psychopharmacology*. 1989; 97:118–122. [PubMed: 2540505]
- Quednow, BB.; Geyer, MA.; Halberstadt, AL. Serotonin and schizophrenia. In: Muller, CP.; Jacobs, B., editors. *Handbook of the Behavioral Neurobiology of Serotonin*. Academic Press; London: 2010. p. 621-636.
- Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci USA*. 1998; 95:14476–14481. [PubMed: 9826725]
- Roth BL, Sheffier DJ, Kroeze WK. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev Drug Discov*. 2004; 3:353–359. [PubMed: 15060530]
- Scorza MC, Castañé A, Bortolozzi A, Artigas F. Clozapine does not require 5-HT_{1A} receptors to block the locomotor hyperactivity induced by MK-801: Clz and MK-801 in KO_{1A} mice. *Neuropharmacology*. 2010; 59:112–120. [PubMed: 20412811]
- Seeman P. Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse*. 1987; 1:133–152. [PubMed: 2905529]
- Stone JM, Davis JM, Leucht S, Pilowsky LS. Cortical dopamine D₂/D₃ receptors are a common site of action for antipsychotic drugs—an original patient data meta-analysis of the SPECT and PET in vivo receptor imaging literature. *Schizophr Bull*. 2009; 35:789–797. [PubMed: 18303092]
- Sumiyoshi T, Bubenikova-Valesova V, Horacek J, Bert B. Serotonin 1A receptors in the pathophysiology of schizophrenia: development of novel cognition-enhancing therapeutics. *Adv Ther*. 2008; 25:1037–1056. [PubMed: 18839076]
- Sumiyoshi T, Matsui M, Nohara S, Yamashita I, Kurachi M, Sumiyoshi C, Jayathilake K, Meltzer HY. Enhancement of cognitive performance in schizophrenia by addition of tandospirone to neuroleptic treatment. *Am J Psychiatry*. 2001; 158:1722–1725. [PubMed: 11579010]
- Swerdlow NR, Mansbach RS, Geyer MA, Pulvirenti L, Koob GF, Braff DL. Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. *Psychopharmacology*. 1990; 100:413–416. [PubMed: 2315438]
- Toth M. 5-HT_{1A} receptor knockout mouse as a genetic model of anxiety. *Eur J Pharmacol*. 2003; 463:177–184. [PubMed: 12600709]
- van den Buuse M. Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. *Schizophr Bull*. 2010; 36:246–270. [PubMed: 19900963]
- Van den Buuse M, Gogos A. Differential effects of antipsychotic drugs on serotonin-1A receptor mediated disruption of prepulse inhibition. *J Pharmacol Exp Ther*. 2007; 320:1224–1236. [PubMed: 17194799]
- van den Buuse M, Wischhof L, Lee RX, Martin S, Karl T. Neuregulin 1 hypomorphic mutant mice: enhanced baseline locomotor activity but normal psychotropic drug-induced hyperlocomotion and prepulse inhibition regulation. *Int J Neuropsychopharmacol*. 2009; 12:1383–1393. [PubMed: 19400983]
- Wood MD, Wren PB. Serotonin–dopamine interactions: implications for the design of novel therapeutic agents for psychiatric disorders. *Prog Brain Res*. 2008; 172:213–230. [PubMed: 18772035]

Yadav PN, Abbas AI, Farrell MS, Setola V, Sciaky N, Huang XP, Kroeze WK, Crawford LK, Piel DA, Keiser MJ, Irwin JJ, Shoichet BK, Deneris ES, Gingrich J, Beck SG, Roth BL. The presynaptic component of the serotonergic system is required for clozapine's efficacy. *Neuropsychopharmacology*. 2011; 36:638–651. [PubMed: 21048700]

Highlights

- Enhanced amphetamine locomotor hyperactivity in 5-HT_{1A} receptor knockout (KO) mice.
- Markedly reduced effect of the hallucinogen 5-MeO-DMT in 5-HT_{1A} receptor KO mice.
- No KO effect on MK-801 locomotor hyperactivity or apomorphine PPI disruption.
- No major changes in the density of dopamine transporters, D₁ or D₂ receptors.
- Important role of 5-HT_{1A} receptors in hallucinations and dopaminergic activity.

Fig. 1.

Psychotropic drug-induced locomotor hyperactivity in wildtype (WT) and 5-HT_{1A} receptor knockout (KO) mice as measured in automated photocell cages. Panel A and B show the effect of injection of saline or 5 mg/kg of amphetamine (Amph, at time =0) in male and female mice, respectively. Panels C and D show the effect of injection of saline or 0.25 mg/kg of MK-801 in male and female mice, respectively. The 5-HT_{1A} receptor knockout mice showed significantly greater hyperactivity after amphetamine treatment compared to WT controls but there was no genotype difference in the response to MK-801. There were 8–12 mice per group.

Fig. 2.

The effect of 10 mg/kg of 5-MeO-DMT (MDMT, top panel) and 1 mg/kg of 8-OH-DPAT (DPAT, bottom panel) on locomotor activity in wildtype (WT) and 5-HT_{1A} receptor knockout (KO) mice as measured in behavioural pattern monitors (BPM). Both drugs significantly reduced locomotor activity in WT mice but had no effect in 5-HT_{1A} receptor knockout mice. There were 6–12 mice per group.

Fig. 3. Prepulse inhibition (PPI) in wildtype (WT) and 5-HT_{1A} receptor knockout (KO) mice. Panels show the effect of 1 or 5 mg/kg of apomorphine (APO1 and APO5, respectively) on startle (top panel), PPI at the 30 ms inter-stimulus interval (ISI, middle panels) and PPI at the 100 ms ISI (bottom panels). Column A represent data in male mice whereas column B represents data in female mice. Treatment with apomorphine dose-dependently reduced PPI in male and female mice but there was no genotype difference.

Total locomotor distance moved of male and female 5-HT_{1A} knockout mice and WT controls at baseline and during 2 h after injection of saline, amphetamine (1 or 5 mg/kg) or MK-801 (0.05 or 0.25 mg/kg). Amphetamine (5 mg/kg) induced greater hyperlocomotion in 5-HT_{1A} knockout mice than in WT controls but there was no genotype difference for MK-801

Table 1

Treatment	Group	Male		Female	
		Baseline	Drug	Baseline	Drug
Cohort 1: Saline	WT	9142 ± 768	7273 ± 1101	10061 ± 523	10359 ± 1401
	5-HT _{1A} KO	8835 ± 535	7535 ± 554	9069 ± 538	9297 ± 1428
	WT	9314 ± 648	8467 ± 1154	9735 ± 739	11363 ± 1774
Amph 1	5-HT _{1A} KO	8102 ± 665	9393 ± 1052	8923 ± 550	12919 ± 1763
	WT	8998 ± 801	31067 ± 2093	10346 ± 821	42350 ± 4380
Amph 5	5-HT _{1A} KO	8842 ± 623	37789 ± 1958	8120 ± 699	51547 ± 4343
	WT	9058 ± 1180	5381 ± 1100	10303 ± 854	9405 ± 1441
Cohort 2: Saline	5-HT _{1A} KO	9420 ± 608	5923 ± 630	8710 ± 739	5943 ± 735
	WT	10171 ± 1008	6723 ± 1122	10010 ± 780	7669 ± 968
MK-801 0.05	5-HT _{1A} KO	7924 ± 863	5580 ± 1098	8771 ± 442	5774 ± 583
	WT	9580 ± 1143	18149 ± 3484	9960 ± 672	19582 ± 1888
MK-801 0.25	5-HT _{1A} KO	8268 ± 884	16394 ± 973	8567 ± 783	14807 ± 1975

There were 8 male WT, 9 female WT, 12 male 5-HT_{1A} knockout and 11 female 5-HT_{1A} knockout mice in cohort 1. There were 9 male WT, 10 female WT, 10 male 5-HT_{1A} knockout and 10 female 5-HT_{1A} knockout mice in cohort 2. For statistical comparisons, see text.

Table 2

DAT levels measured by the density of [^3H]GBR12935 binding in fmol/mg ETE in forebrain sections of male and female 5-HT_{1A} knockout mice and WT controls. There were no differences between male or female mice or between the genotypes. However, a significant region $\times$ genotype interaction reflected that binding tended to be higher in the caudate nucleus than in the nucleus accumbens of WT mice, whereas this difference was not seen in 5-HT_{1A} KO mice.

Group/Brain regions	N. Acc. shell	N. Acc. core	Caudate n.
Male WT controls	33.91 $\pm$ 2.38	32.35 $\pm$ 2.38	41.22 $\pm$ 1.32
Female WT controls	34.48 $\pm$ 3.77	29.59 $\pm$ 3.93	40.56 $\pm$ 4.90
Male 5-HT _{1A} KO	39.92 $\pm$ 3.43	40.49 $\pm$ 4.42	43.45 $\pm$ 4.28
Female 5-HT _{1A} KO	34.33 $\pm$ 1.38	33.21 $\pm$ 1.77	34.34 $\pm$ 3.04

There were 9 male WT, 7 female WT, 7 male 5-HT_{1A} knockout and 5 female 5-HT_{1A} knockout mice. For statistical comparisons, see text.

Table 3

Dopamine D₂ receptor levels measured by the density of [³H]YM-09151 binding in fmol/mg ETE in forebrain sections of male and female 5-HT_{1A} knockout mice and WT controls. Binding densities were significantly lower in female WT controls than in male WT controls, but there was no sex difference in 5-HT_{1A} KO mice.

Group/Brain regions	N. Acc. shell	N. Acc. core	Caudate n.
Male WT controls	59.42 ± 3.96	61.12 ± 2.73	58.51 ± 1.92
Female WT controls	44.77 ± 3.41	43.06 ± 2.53	43.54 ± 2.07
Male 5-HT _{1A} KO	50.22 ± 5.06	49.06 ± 4.41	48.72 ± 3.86
Female 5-HT _{1A} KO	51.27 ± 7.61	52.33 ± 6.69	57.55 ± 6.32

There were 6 male WT, 7 female WT, 7 male 5-HT_{1A} knockout and 7 female 5-HT_{1A} knockout mice. For statistical comparisons, see text.

Table 4

Dopamine D₁ receptor levels measured by the density of [³H]SCH23390 binding in fmol/mg ETE in forebrain sections of male and female 5-HT_{1A} knockout mice and WT controls. Binding densities were significantly lower in female WT controls than in male WT controls, but there was no sex difference in 5-HT_{1A} KO mice.

Group/Brain regions	N. Acc. shell	N. Acc. core	Caudate n.
Male WT controls	150.3 ± 7.6	128.7 ± 7.2	147.6 ± 10.8
Female WT controls	139.5 ± 24.6	118.9 ± 24.2	164.2 ± 25.7
Male 5-HT _{1A} KO	163.4 ± 19.8	135.1 ± 16.5	166.2 ± 19.4
Female 5-HT _{1A} KO	154.7 ± 16.3	144.8 ± 20.1	158.3 ± 15.0

There were 8 male WT, 6 female WT, 5 male 5-HT_{1A} knockout and 6 female 5-HT_{1A} knockout mice. For statistical comparisons, see text.