

Mapping of CBV changes in 5-HT_{1A} terminal fields by functional MRI in the mouse brain

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Abstract

Visualization of brain activity in humans and animals using functional magnetic resonance imaging (fMRI) is an established method for translational neuropsychopharmacology. It is useful to study the activity of defined brain structures, however it requires further refinement to allow more specific cellular analyses, like for instance, the activity of selected pools of brain cells. Here, we investigated brain activity in serotonergic pathways in the adult mouse brain by using acute pharmacological challenge of 5-hydroxytryptamine (5-HT) 1A receptors. We show that administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT prompts a dose-dependent reduction in local cerebral blood volume (CBV) in brain areas rich in neurons expressing post-synaptic 5-HT_{1A} receptor, including the prefrontal cortex, hippocampus and amygdalar nuclei. Region-specific inhibition of the response by co-injection of 8-OH-DPAT with the selective 5-HT_{1A} receptor antagonist WAY-100635, or in 5-HT_{1A} knock-out mice, suggests that 5-HT_{1A} receptors are the

Abbreviations: 5-HT-R, 5-hydroxy-tryptamine (serotonergic) receptor; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin; BLA, basolateral amygdala; BM, basomedial amygdala; CBV, cerebral blood volume; Cg, cingulate cortex; DHC, dorsal hippocampus; DR, dorsal raphe nucleus; fMRI, functional magnetic resonance imaging; LA, lateral amygdala; LS, lateral septum; PAG, periaqueductal gray; PET, positron emission tomography; RF, radiofrequency; ROI, region-of-interest; SNR, signal-to-noise ratio; SE-RARE, Spin-echo rapid acquisition with relaxation enhancement technique; tcpCO₂, transcutaneously assessed partial pressure of carbon dioxide; VHC, ventral hippocampus; WAY-10063, N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl)-cyclohexane carboxamide.

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primary targets of the agonist. Overall, the data demonstrate the feasibility of mapping region-specific serotonergic transmission in the adult mouse brain *in vivo* by non-invasive fMRI. The method opens novel perspectives for investigating 5-HT_{1A} receptor functions in mouse models of human pathologies resulting from a dysfunction of the 5-HT_{1A} receptor or the serotonergic system, including depression and anxiety.

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1. Introduction

Alterations in serotonin (5-hydroxytryptamine; 5-HT) neurotransmission have been implicated in the pathophysiology of several psychiatric diseases, including depression and anxiety disorders (Garner et al., 2009; Graeff, 2002). The modulatory monoamine serotonin binds to specific transmembrane receptors that belong to a large family (Hannon and Hoyer, 2008). One of the best-characterized receptors is the 5-HT_{1A} receptor which is coupled to inhibitory G-proteins, and is implicated in the etiology of depression (Blier and Ward, 2003; Neumeister et al., 2004; Albert and Lemonde, 2004). In the mammalian brain, 5-HT_{1A} receptors are localized both, presynaptically as somatodendritic autoreceptors in the raphe nucleus, and postsynaptically in prefrontal and frontal cortex, septal nuclei, periaqueductal gray and limbic areas such as hippocampus and amygdala (Hoyer et al., 1986; Laporte et al., 1994; Bockaert et al., 2006). This pre- versus post-synaptic distinction is functionally important because pre- and post-synaptic receptor populations mediate different responses. Activation of 5-HT_{1A} autoreceptors by administration of a 5-HT_{1A} receptor agonist such as 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, aminotetralin) (Arvidsson et al., 1981), decreases the firing rate of serotonergic neurons in hippocampus and cortex, and reduces the level of 5-HT release in these areas (Sprouse and Aghajanian, 1988; Hjorth and Sharp, 1991). In contrast, post-synaptic receptors play a modulating role by regulating the activity of non-serotonergic neurons in 5-HT terminal fields. Under physiological conditions, neurons in the raphe nucleus remain under the functional control of projection neurons located in the medial prefrontal cortex, in part through post-synaptic 5-HT_{1A} receptors (Celada et al., 2002).

In vivo functional measurement of 5-HT_{1A} receptor activity is an attractive method to evaluate the role of 5-HT_{1A} receptors in the pathology of psychiatric disorders. For instance, the density of 5-HT_{1A} receptors in brain regions implicated in emotional regulation and response to stress was imaged in patients with depression or anxiety disorders using positron-emission-tomography (PET), and was found to be decreased (Sullivan et al., 2005; Lanzenberger et al., 2007; Kumar and Mann, 2007; Hirvonen et al., 2008; Savitz et al., 2009). However, analogous PET studies in mouse models of psychiatric disorders are less established. So far, only the fluorinated tracers [¹⁸F]FCWAY and [¹⁸F]FPWAY, analogues of the 5-HT_{1A} receptor antagonist WAY100635 were evaluated *ex vivo* in mice (Jagoda et al., 2006). In general, meaningful small animal PET experiments are still limited by the availability of suitable PET tracers and a spatial resolution of >1 mm³. Furthermore, PET measurements only provide information on a given receptor expression while fMRI measures the functional consequences of the receptor stimulation which can be achieved at much higher resolution compared to PET.

Pharmacological fMRI is a powerful method that allows monitoring of the acute effects of specific receptor ligands (agonists or antagonists) on brain activity. A large majority of pharmacological fMRI studies in animals have been carried out in rats and analyzed in regards to both the magnitude and spatial extent of fMRI responses for dopaminergic (Chen et al., 1997), GABAergic (Reese et al., 2000), glutamatergic (Houston et al., 2001; Jones et al., 2005), or serotonergic (Houston et al., 2001) neurotransmission. The concept was also successfully applied to animal models of neurodegenerative and psychiatric dysfunctions, and has become a common method for the preclinical characterization of CNS drugs (Rudin et al., 2003; Borsook et al., 2006; van der Linden et al., 2007; Martin and Sibson, 2008). For the 5-HT system, most fMRI studies (Houston et al., 2001; Stark et al., 2006; Hackler et al., 2007; Stark et al., 2008) have focused on the 5-HT_{2C} receptor using the 5-HT_{1B/2C} receptor agonist *meta*-chlorophenylpiperazine (*m*-CPP), essentially because this receptor is a major target for novel potential anxiolytic drugs (Wood, 2003). Only one study examined the 5-HT_{1A} receptor so far. In this study, the acute administration of the agonist 8-OH-DPAT in rat was shown to decrease CBV in several brain areas, in particular the hippocampus and septum (Scanley et al., 2001).

Pharmacological fMRI in the mouse has not yet often been used despite the fact that genetically engineered mice are powerful models of brain disorders. The feasibility of fMRI in the mouse has nonetheless already been demonstrated in transgenic models of Alzheimer's disease (Mueggler et al., 2002, 2003) and schizophrenia (Kuriwaki et al., 2004). However, it is still limited by the requirement of a higher spatial resolution due to smaller brain size and for an increased sensitivity of signal detection. The use of cryogenic radio-frequency (RF) detector devices for mouse brain studies has emerged as a cost effective solution for increasing such sensitivity. It allows a 2–2.5 fold gain when compared to RF receiver operating at room temperature (Ratering et al., 2008; Balthes et al., 2009). Additionally, compared to fMRI studies in the rat, the experimental conditions needed to provide stable physiological parameters for mouse studies are demanding (Mueggler, 2006).

In this study, our aim was to show the feasibility of monitoring CBV response, as a representation of 5-HT_{1A} receptor-mediated neuronal activity, after challenge with the 5-HT_{1A} receptor agonist 8-OH-DPAT in the adult mouse brain *in vivo*. We aimed at detecting distinct decreases in CBV correlating with the region-specific expression of the 5-HT_{1A} receptor. The specificity of the pharmacological manipulation was validated using the 5-HT_{1A} receptor antagonist N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl)-cyclohexane carboxamide (WAY-10063) (Laporte et al., 1994), and a knock-out mouse model deficient for 5-HT_{1A} receptor.

2. Experimental procedures

2.1. Animals

Male C57Bl/6 mice (3–4 months of age) of 22–25 g body weight were used. Animals were housed under a normal light/dark cycle (12:12 h) with standard rodent chow and tap water *ad libitum*. Complementary proof-of-concept studies were carried out in homozygous (HOM) and heterozygous (HET) mice lacking the 5-HT_{1A} receptor (5-HT_{1A} knock-out), and corresponding wild type littermates (WT), obtained from EMBL, Monterotondo, Italy (courtesy C. Gross, Gross et al., 2002; Ramboz et al., 1998). Genotyping was performed using standard protocol and primers for 5-HT_{1A} (XZAU 5'-CAG TCT CTA GAT CCC CTC CCT T (common primer), XZTL 5'-AAG GGC AAA AGT GAG TAT GGT G, HTR1A 5'-GGG CGT CCT CTT GTT CAC GTA G).

2.2. Animal preparation for fMRI

For imaging, animals were anesthetized with isoflurane® (Abbott, Cham, Switzerland), endotracheally intubated with a polyethylene (PE) tube (inner/outer diameter: 0.58/0.96 mm, Portex®, Smith Medical International Ltd. Kent UK), placed on a cradle made from Plexiglas with in-built warm water circulation, and artificially ventilated according to (Mueggler et al., 2001). Body temperature was monitored using a rectal probe coupled to a fluoroptic module (QUASYS AG, Cham, Schweiz) and kept constant at 36.5 ± 0.5 °C. Ear bars secured reproducible positioning and reduced motion artifacts. The tail vein was cannulated with a 30G needle (0.3 mm × 13 mm, BD Microlance, Drogheda, Ireland) for drug administration. A single dose of 15 mg/kg of the neuromuscular blocker gallamine triethiodide (Sigma-Aldrich, Steinheim, Germany) was injected and the isoflurane® level was reduced to 1.5% in air/oxygen (3:1). To control for potentially confounding drug-induced systemic effects a separate series of off-line (bench) experiments were carried out assessing pCO₂ measurements during administration of 8-OH-DPAT, using the same protocol as for fMRI series. In addition the animal's upper hindlimb was shaved and depilated (veet, Reckit Benckiser, UK) to position the fixation ring of the electrode of the transcutaneously operating non-invasive blood gas monitoring device TCM4 (Radiometer Medical ApS, Denmark). All experiments were carried out in strict adherence to the Swiss Law for Animal Protection, approved by the veterinary office of the canton of Zurich, Switzerland.

2.3. Imaging

Experiments were performed using a Pharmascan MR system 47/16 (Bruker BioSpin GmbH, Karlsruhe, Germany) equipped with a 200 MHz cryogenic receive radiofrequency (RF) probe (Ratering et al., 2008). Prior to fMRI, anatomical images were recorded using a multi-slice RARE (rapid acquisition with relaxation enhancement technique (Hennig et al., 1986)) spin echo (SE) sequence with an effective echo time $TE_{\text{eff}} = 10.4$ ms, a repetitions time $TR = 900$ ms, an echo spacing $TE = 10.4$ ms and RARE-factor 5. Further acquisition parameters were field-of-view $FOV = 20 \times 13$ mm², matrix dimensions $MD = 200 \times 130$, slice thickness $STH = 0.7$ mm (yielding a voxel dimension of $100 \times 100 \times 700$ μm³), and interslice distance $ISD = 1.2$ mm, number of slices $NSL = 8$. Reproducible slice positions across animals were insured by operator-interactive positioning of the most posterior slice in the central fissure -5.34 mm relative to the Bregma. The fMRI protocol consisted of a RARE sequence with a spatial resolution of $156 \times 156 \times 700$ μm³ and a temporal resolution of 40 s/multislice data set; Further parameters were $TE_{\text{eff}}/TR = 80.2/2500$ ms, $FOV = 20 \times 13$ mm², $MD = 128 \times 128$, $STH = 0.7$ mm, $ISD = 1.2$ mm, $NSL = 8$. fMRI data were acquired over three phases: at first, 8 images (S_{pre}) were acquired prior to the intravenous (i.v.) administration of the contrast agent (Endorem®, 55 mg Fe/kg, Laboratoire Guerbet SA,

Paris). After a period of 15 min, to allow the contrast agent to reach steady-state concentration in plasma, a second series of 35 post-contrast images (yielding the average signal $S(0)$) were acquired prior to drug administration, followed by the last series of 51 images ($S(t)$).

2.4. Pharmacological fMRI paradigm

After the 35th image, 5-HT_{1A} receptor ligand(s) or a control vehicle (saline) was administered as i.v. bolus injection (volume 2 ml/kg). For the dose-dependence study, the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (OH-DPAT) was given at doses of 0.02 ($N = 5$), 0.05 ($N = 7$), 0.075 ($N = 6$) and 0.1 mg/kg ($N = 7$) or vehicle ($N = 5$), respectively. For control (off-line) experiments enabling monitoring of pCO₂ values the same infusion protocol was used with 8-OH-DPAT administered at a dose of 0.1 mg/kg ($N = 6$). The specificity of effects was evaluated by co-injecting OH-DPAT at 0.1 mg/kg with the potent 5-HT_{1A} receptor antagonist WAY-100635 (0.54 mg/kg) ($N = 7$). For experiments using 5-HT_{1A} knockout mice, OH-DPAT at a dose of 0.1 mg/kg has been used (HET: $N = 10$ HOM: $N = 2$, WT: $N = 10$).

2.5. Data analyses and statistics

Data analyses were carried out using Biomap (Novartis Institute for Biomedical Research, M. Rausch). To eliminate overall signal drift over time due to wash-out effects of the contrast agent, the temporal signal profile for each voxel was first detrended by a simple linear regression derived from baseline interval. Changes in CBV (% of baseline values, $\Delta CBV_{\%}$) were computed for selected regions of interest (ROIs) according to $\Delta CBV_{\%}(t) = \frac{\ln\{S(t)/S_0\}}{\ln\{S_0/S_{pre}\}} \cdot 100$

ROIs were defined for cingulate cortex (Cg, on image slice +1.94 mm relative to the Bregma), lateral septum (LS, +0.74 mm), lateral (LA, -1.82 mm), basolateral (BLA, -1.82 mm) and basomedial amygdala (BM, -1.82 mm), dorsal raphe nucleus (DR, -4.04 mm) and periaqueductal gray (PAG, -4.04 mm). To contribute to the multiple functions of the hippocampus in the direction of functional segregation along its long-axis, we distinguished between dorsal (DHC -1.82 mm) and ventral (VHC -3.08 mm) hippocampus. Color-coded $\Delta CBV_{\%}$ maps were computed for the periods -5 to 0 min (immediately prior to drug injection = baseline map) and 5–10 min following 8-OH-DPAT injection (representing stimulation period = activity map) by averaging 8 functional images followed by a first-order low-pass filter. Dose dependent effects of 5-HT_{1A} ligands in C57B6 mice were tested by comparing mean $\Delta CBV_{\%}$ values for the interval 0–26 min of the temporal profiles ($t = 0$ being the time point of drug administration) using ANOVA (Statview5, SAS Institute Inc., Cary, NC). To test for a genotype effect, the temporal profile (0–26 min) of $\Delta CBV_{\%}$ was analyzed using repeated measurement ANOVA followed by Fisher's PLSD post-hoc test with a significance level of $p < 0.05$ when appropriate. Results are expressed as mean ± SEM.

3. Results

3.1. CBV effect of acute 8-OH-DPAT administration

In C57Bl/6 mice, a dose-dependent and region-specific decrease in $\Delta CBV_{\%}$, was observed following the administration of the 5-HT_{1A} agonist 8-OH-DPAT (Fig. 1). The profile of $\Delta CBV_{\%}$ reflected the distribution of 5-HT_{1A} receptors in the brain (Barnes and Sharp, 1999). Anatomical references and ROIs used for quantitative analyses of $CBV_{\%}$ changes relative to the Bregma were defined on the basis of a mouse brain atlas (Fig. 1A, adapted from Paxinos and Franklin, 2001) and corresponding

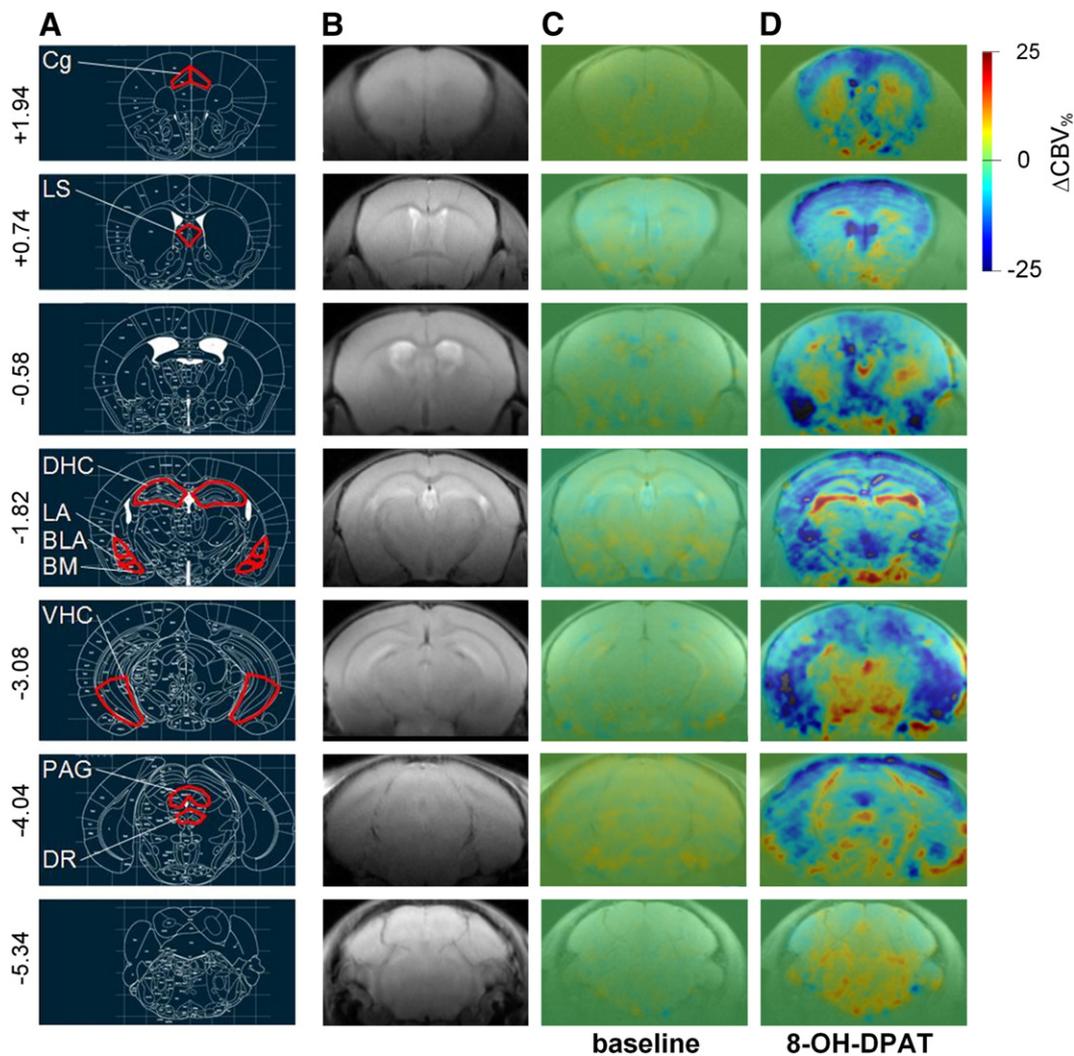


Figure 1 Anatomical reference slices and $\Delta\text{CBV}_{\%}$ maps superimposed on anatomical MR images. (A) Coronal sections from a mouse brain atlas (adapted from Paxinos and Franklin, 2001), indicating the position of fMRI images (+1.94 to -5.34 mm) relative to Bregma. Brain structures used in the statistical analysis (ROIs) are outlined in red. (B) Corresponding anatomical SE-RARE image. (C) Pseudo-colored and low-pass filtered activation maps superimposed onto the corresponding anatomical images. Maps were generated by integration of 8 baseline images for the interval -5 to 0 min (immediately prior to drug injection). (D) Activity maps ($\Delta\text{CBV}_{\%}$) representing stimulation period 5 – 10 min following injection of 0.1 mg/kg 8-OH-DPAT. Pronounced CBV changes can be observed in prefrontal cortex, septum, amygdala, dorsal and ventral parts of the hippocampus, entorhinal/piriform cortex and periaqueductal gray. Abbreviations: BLA, basolateral amygdala; BM, basomedial amygdala; Cg, cingulate cortex; DHC, dorsal hippocampus; DR, dorsal raphe nucleus, LA, lateral amygdala; LS, lateral septum; PAG, periaqueductal gray.

MR structural images (Fig. 1B). To improve signal-to-noise ratio, pseudo-colored $\Delta\text{CBV}_{\%}$ maps were generated by averaging the respective values over 8 consecutive images (SNR). Corresponding maps are displayed as an overlay onto the matching high-resolution reference image at baseline (Fig. 1C, before drug injection) and during the stimulation period (5 – 10 min after 8-OH-DPAT injection, Fig. 1D). A pronounced decrease in $\text{CBV}_{\%}$ was observed in cingulate cortex (Cg), lateral septum (LS), amygdala, ventral hippocampus (VHC), and periaqueductal grey (PAG) as well as in the entorhinal and piriform cortex. In the striatum, a minor increase in $\text{CBV}_{\%}$ was observed, while $\text{CBV}_{\%}$ was not changed in brainstem structures.

3.2. Dose-dependent $\text{CBV}_{\%}$ response in terminal fields of 5-HT_{1A} neurons and inhibition by WAY-100635

The dose-dependence of $\Delta\text{CBV}_{\%}$ response to 8-OH-DPAT was evaluated for a dose range $0.02 < \text{dose} < 0.1$ mg/kg (Fig. 2A). In Cg, intravenous administration of 8-OH-DPAT resulted in a transient decrease in $\text{CBV}_{\%}$ of 20.7% (± 3.2) for 0.1 mg/kg, 12.6% (± 3.9) for 0.075 mg/kg and 7.7% (± 3.1) for 0.05 mg/kg. However at the lowest dose (0.02 mg/kg), 8-OH-DPAT caused a slight but steady increase in $\text{CBV}_{\%}$ throughout the measurement interval, up to $+11.7\%$ (± 1.7) about 30 min following drug

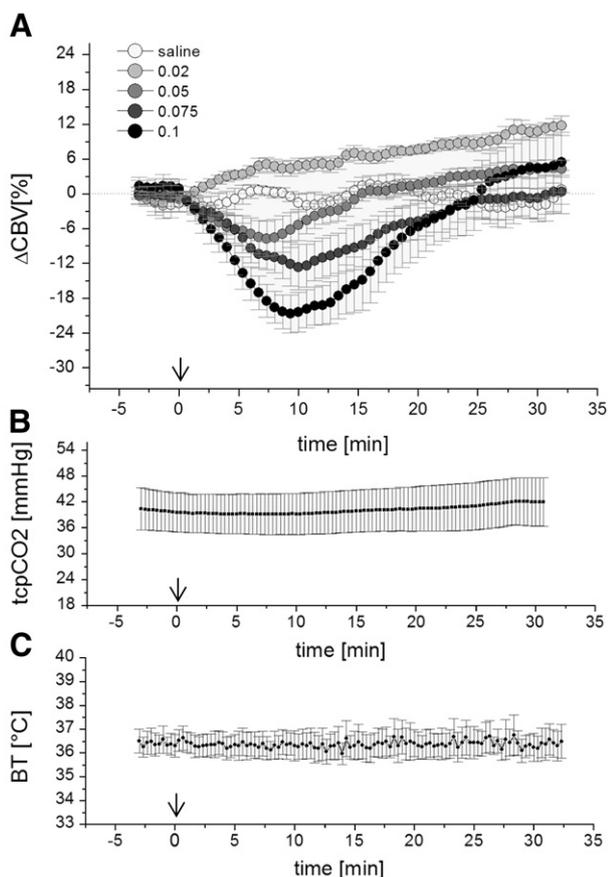


Figure 2 Temporal CBV profile for all dosages used and data from physiological monitoring (body temperature and transcutaneously assessed pCO_2 values) for the highest dose (0.1 mg/kg) of 8-OH-DPAT. (A) $\Delta CBV\%$ profile in Cg of C57Bl/6 control mice after systemic injection of 8-OH-DPAT (indicated by arrow) at 4 different doses and after saline injection. A clear dose-dependent effect was observed: $\Delta CBV\%$ was transiently decreased at high doses of 8-OH-DPAT (0.05 and 0.1 mg/kg) while it was steadily increased at the lowest dose (0.02 mg/kg) throughout the observation period. (B) Temporal profile of body temperature (BT) over the time-course of CBV assessment during infusion of 8-OH-DPAT at a dose of 0.1 mg/kg ($N=6$). Data were derived from on-line monitoring using rectal probe coupled to a fluoroptic module. (C) Time-course of transcutaneous CO_2 values ($tcpCO_2$) during infusion of 8-OH-DPAT at a dose of 0.1 mg/kg ($N=6$) assessed off-line in a separate series of experiments under identical conditions as for fMRI series ($N=6$). Data were obtained using non-invasive blood gas monitoring device TCM4. All values are given as mean \pm SEM.

administration. Within the placebo group (saline injection, 1 ml/kg), no change in $CBV\%$ was observed. A similar transient and dose-dependent effect of 8-OH-DPAT was also measured in VHC and amygdalar structures (BLA, LA and BM), and in PAG. No significant change in $CBV\%$ was detected in the dorsal raphe nucleus (DR). Fig. 2A and B show data from physiological monitoring of body temperature and transcutaneously assessed pCO_2 ($tcpCO_2$) during administration of the highest dose of 8-OH-DPAT (0.1 mg/kg). During the time course of the experiment, values were within physiological range (mean value for body temperature, 36.4 ± 0.2 °C; pCO_2 , 39.1 ± 2.2 mmHg) and no

alteration during or after infusion of the 5-HT_{1A} agonist was observed.

For statistical analyses of $\Delta CBV\%$, values (in%) were compared for the time interval $0 < t < 26$ min for all doses using ANOVAs. This time interval was chosen because the CBV curve after injection of 0.1 mg/kg 8-OH-DPAT reached baseline values after 26 ± 2 min. The dependence of these values on the 8-OH-DPAT dose is illustrated in Fig. 3 for ROIs in Cg, LA, VHC, PAG and DR. Of these five structures, all except DR showed a main dose effect on $\Delta CBV\%$ (Table 1). CBV was reduced in a dose-dependent manner in Cg with values for 0.075 and 0.1 mg/kg 8-OH-DPAT being significantly different from saline-treated mice. For both LA and VHC, $\Delta CBV\%$ values for 0.05, 0.075 and 0.1 mg/kg 8-OH-DPAT were significantly different from those for saline (SAL) (all $p < 0.008$ for LA, $p < 0.05$ for VHC) and 0.02 mg/kg 8-OH-DPAT (all $p < 0.006$ for LA, $p < 0.002$ for VHC). In PAG, only the $\Delta CBV\%$ values for 0.075 and 0.1 mg/kg 8-OH-DPAT were significantly different from SAL (all $p < 0.001$) and from 0.02 mg/kg 8-OH-DPAT (all $p < 0.0001$, Fig. 3). No main effect of dose was observed in DR (Table 1). While the lowest dose of 8-OH-DPAT (0.02 mg/kg) yielded a CBV increase of $3 \pm 2\%$, higher doses (0.05–0.1 mg/kg) changes CBV in the range of $-1\% < \Delta CBV\% < 1\%$.

To assess the specificity of the response, we co-administered 8-OH-DPAT (0.1 mg/kg) with the specific 5-HT_{1A} antagonist WAY-100635 (0.54 mg/kg). The results suggested that the effect of 8-OH-DPAT on $CBV\%$ can be attributed to 5-HT_{1A} activation (Fig. 3, Table 2). In Cg, co-administration abolished the decrease in $CBV\%$ induced by the agonist ($F(3, 16) = 8.5$, $p < 0.002$). A similar antagonistic effect was observed in the VHC ($F(3, 16) = 4.5$, $p < 0.02$) and in LA ($F(3, 17) = 5.8$, $p < 0.007$). For these regions, co-injection of 8-OH-DPAT and WAY 100635 significantly reduced CBV response when compared to the response with 8-OH-DPAT alone (Table 2). It should be noted nonetheless that for the doses used, the change in CBV elicited by the agonist could not be fully abolished by the antagonist, most likely due to a different degree of receptor activation and blockade by the drugs. Thus, while WAY-100635 alone at 0.54 mg/kg slightly decreased CBV (4%) in Cg, (data not shown), its co-injection with 8-OH-DPAT induced a $7 \pm 1\%$ decrease as compared to $14 \pm 3\%$ for 8-OH-DPAT alone. Overall however, no significant difference in $\Delta CBV\%$ was observed following the injection of saline or WAY-100635 alone except in PAG (Table 2). Furthermore, no significant main effect of dose was found for DR ($F(3, 16) = 0.08$, $p = 0.971$).

3.3. 5-HT_{1A} knockout mice have altered $CBV\%$ response upon stimulation with 8-OH-DPAT

To further confirm the specificity of 5-HT_{1A} receptor activation by 8-OH-DPAT, we evaluated its effect in mice lacking the 5-HT_{1A} receptor (HET and HOM knock-out mice and WT littermates). In WT littermates, a decrease in $CBV\%$ similar to that observed in C57Bl/6 mice used for the dose-dependent study was observed (see Fig. 4). A significant difference between HET or HOM, and WT in Cg was observed following variance analysis of the temporal profile (0–26 min) of $\Delta CBV\%$ (main effect of genotype $F(2, 20) = 5.32$, $p < 0.02$, PLSD WT vs. HET ns, WT vs. HOM $p < 0.05$ and HET vs. HOM $p < 0.03$, respectively, Fisher's PLSD). A negative $\Delta CBV\%$ response was observed in HET, but tended to be weaker than in WT animals. HOM mice in contrast,

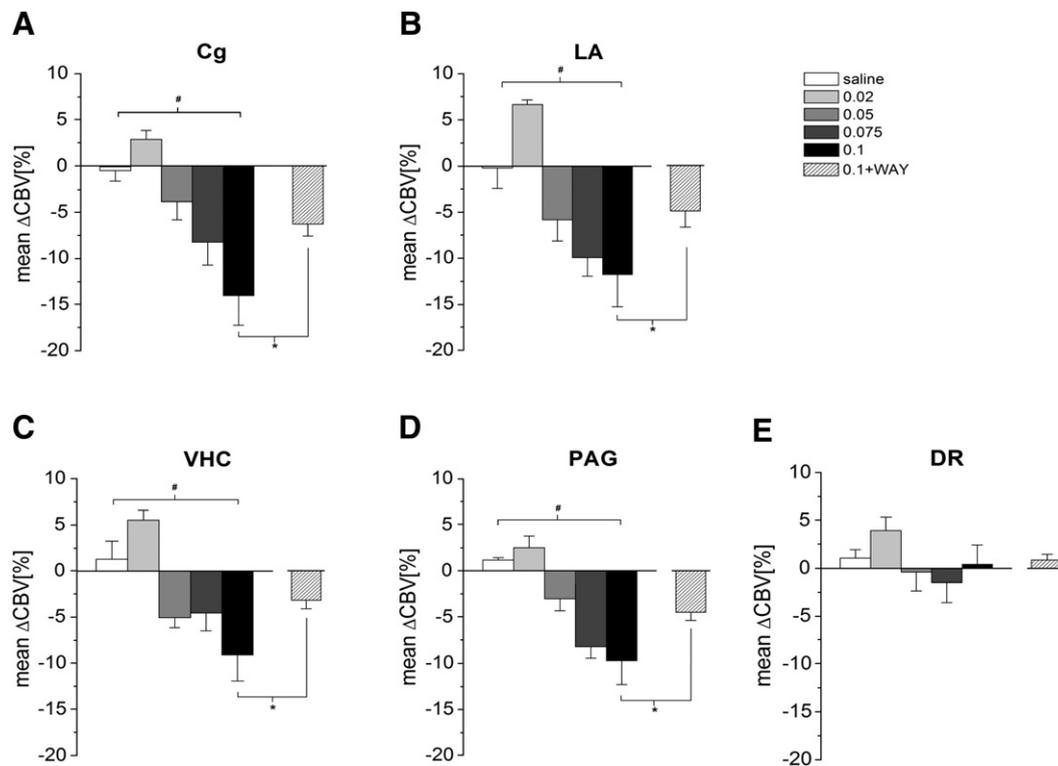


Figure 3 Integrated $\Delta\text{CBV}\%$ changes (integration period 0–26 min) after injection of saline, 8-OH-DPAT (0.02, 0.05, 0.075 and 0.1 mg/kg) and after co-injection 8-OH-DPAT (0.1 mg/kg) with WAY-100635 (0.54 mg/kg). $\Delta\text{CBV}\%$ values were calculated from ROIs in cingulate cortex (A), lateral amygdala (B), ventral hippocampus (C), periaqueductal gray (D) and dorsal raphe nucleus (E). Dose-dependent effects were tested by comparison of mean $\Delta\text{CBV}\%$ of the temporal profile (0–26 min) using ANOVA. Values are given as mean \pm SEM. Data reveal a significant dose-effects for all structures except the dorsal raphe nucleus ($\# = p < 0.05$). Statistical comparison of single injection 8-OH-DPAT and coinjection of 8-OH-DPAT and WAY-100635 indicates the antagonist properties of WAY-100635 and the specificity of the 5-HT_{1A} activation by 8-OH-DPAT ($* = p < 0.05$; Fisher's PLSD).

displayed a significantly different pattern, and showed a region-specific minor increase in $\text{CBV}\%$ following stimulation with the 5-HT_{1A} agonist. For example in LA, $\text{CBV}\%$ was decreased in WT and HET but not in HOM mice, as reflected by a significant main effect of genotype ($F(2, 20) F = 8.36, p < 0.003$ and subsequent significant post-hoc comparisons (WT vs. HET ns, WT vs. HOM $p < 0.0007$ and HET vs. HOM $p < 0.005$). In other brain regions including PAG, a slight reduction in $\text{CBV}\%$ was similarly observed in WT and HET, but an opposite response in HOM mice (main effect of genotype $F(2,21) = 11.68, p < 0.0005$). No significant genotype-specific effect was observed in DR.

4. Discussion

This study shows that administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT reduces $\text{CBV}\%$ in a dose-dependent and

region-specific manner in the adult mouse brain as revealed by fMRI. The drug induced pronounced changes in relative $\text{CBV}\%$ in brain regions expressing a high level of post-synaptic 5-HT_{1A} receptor (Barnes and Sharp, 1999), such as anterior cingulate cortex, hippocampus (with effects in dorsal larger than in ventral hippocampus), subiculum, amygdala nuclei and lateral septum. The 5-HT_{1A} receptor agonist is

Table 1 Statistical values derived from ANOVA showing main effect of the dose of 8-OH-DPAT (saline, 0.02, 0.05, 0.075 and 0.1 mg/kg) on $\Delta\text{CBV}\%$ for all regions except DR.

	Cg	LA	VHC	PAG	DR
$F(4,12)$	9.72	11.59	8.85	11.55	1.2
p	0.0001	<0.0001	0.0002	<0.0001	0.34

Table 2 Statistical values derived Fisher's PLSD post-hoc comparisons ($F(3,16)$). With significant p -values highlighted in bold. DR showed no main effect of dose and was not tested further.

	Cg	LA	VHC	PAG
SAL vs 8-OH-DPAT	0.0007	0.0008	0.0026	<0.0001
SAL vs 8-OH-DPAT + WAY100635	0.0995	0.86	0.1589	0.0267
SAL vs WAY100635	0.9405	0.06	0.1682	0.0427
8-OH-DPAT vs 8-OH-DPAT + WAY100635	0.02	0.0211	0.0461	0.0178
8-OH-DPAT vs WAY100635	0.0008	0.052	0.0428	0.0039
WAY100635 vs 8-OH-DPAT + WAY100635	0.1145	0.7434	0.972	0.549

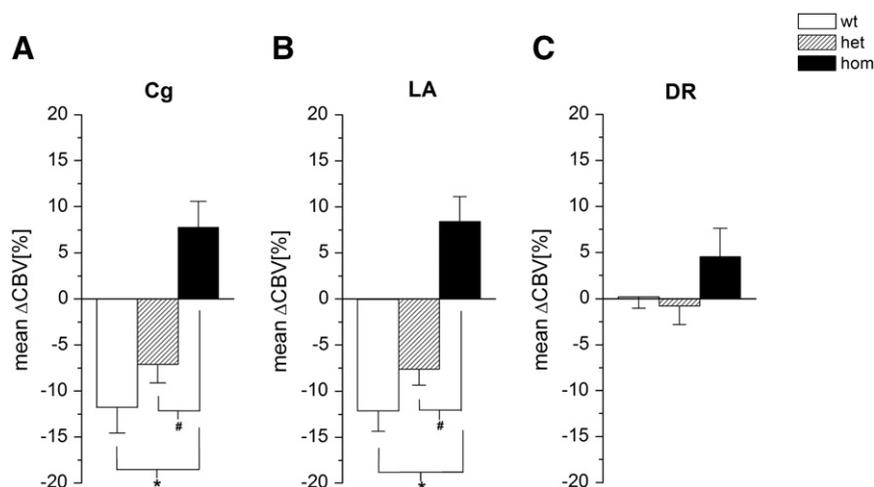


Figure 4 Δ CBV% changes after injection of 8-OH-DPAT (0.1 mg/kg) in 5-HT_{1A} receptor knockout and control mice. Integral Δ CBV% values (for period 0–26 min) have been depicted for ROIs in cingulate cortex (A), lateral amygdala (B), and dorsal raphe nucleus (C). Genotype specific effect was tested by comparison of the temporal profile (0–26 min) using ANOVA with repeated measurements. Values are given as mean \pm SEM. The CBV response observed in homozygous 5-HT_{1A} receptor knockout mice is significantly different compared to wild type (* = $p < 0.05$, Fisher's PLSD) and heterozygous animals (# = $p < 0.05$).

known to reduce the level of cyclic AMP (cAMP) and secondary messenger signaling, and leads to an inhibition of cell depolarization (Hannon and Hoyer, 2008). This in turn, results in distinct metabolic changes such as a pronounced decrease in glucose consumption in territories of 5-HT_{1A} innervation (Freo, 1996), which translate into a hemodynamic response that can be detected by fMRI.

The observation that alterations in CBV response induced by 8-OH-DPAT can be reversed by the 5-HT_{1A} antagonist WAY-100635 strongly suggests that the effect is mediated essentially by the 5-HT_{1A} receptor. Both 8-OH-DPAT and WAY-100635 have been shown to selectively bind to 5-HT_{1A} receptors in the brain *in vivo* following i.v. administration in mice (Fletcher et al., 1993). The decrease in CBV in the areas containing 5-HT innervation might occur via several potential mechanisms. It may be mediated by activation of somatodentritic 5-HT_{1A} autoreceptors located in DR that might decrease the firing rate of raphe 5-HT neurons (Blier et al., 1998), and/or decrease 5-HT release in projection territories such as prefrontal cortex, hippocampus, amygdala and septum (Hillegaart et al., 1990). Alternatively, 8-OH-DPAT might also act directly on post-synaptic 5-HT_{1A} receptors located on non-serotonergic neurons in projection areas. In turn, the inhibitory effect of WAY-100635 may also be mediated by either presynaptic or post-synaptic receptors, or may be the result of both types of receptors. Moreover, in ascending projections originating in the raphe nucleus, the 5-HT_{1A} receptor is present as a hetero-receptor on non-serotonergic neurons, for instance on glutamatergic neurons which are then activated (Czyrak et al., 2003; Chessell et al., 1993). While the observed CBV response clearly demonstrates activation of a complex neuronal network within 5-HT_{1A} innervated areas, it does not provide sufficient information for elucidating the underlying mechanism.

The absence of a CBV response observed in the caudate putamen after 5-HT_{1A} stimulation is in line with the low density of 5-HT_{1A} receptors in this area. This density is indeed low compared to regions of the limbic system (i.e.

amygdala, hippocampus, entorhinal cortex) (Lanfumeu and Hamon, 2000; Laporte et al., 1994; Barnes and Sharp, 1999). Likewise, brain areas caudal to the raphe nucleus such as the dorsal pons, tectum or colliculus, or in the medial midbrain nuclei express a low level of 5-HT_{1A} receptor, and did not show any CBV response (Fig. 1D).

It was reported that DR has only approximately 11,000 5-HT neurons in the rat (Descarries et al., 1982). Stimulation of these receptors is likely to prompt only weak local metabolic and hemodynamic responses that may be too small to be detected by fMRI. In an earlier study in rat, this aspect was not addressed since structures in the raphe nucleus were not analyzed (Scanley et al., 2001). In the present study, this point may explain the discrepancy between our fMRI data and previous electrophysiological studies showing activation of 5-HT_{1A} neurons in the raphe nucleus with a similar dose of 8-OH-DPAT (Martin-Ruiz and Ugedo, 2001). In addition, partial volume effects might compromise the detection of a CBV response. Slices used in the fMRI study are thick (0.7 mm) compared to the dimensions of the raphe nucleus (~0.35 mm anterior–posterior dimension), thus the fMRI signal is diminished or diluted by the averaging of the signal from responding and non-responding brain regions across the slice. This limitation has also been observed in *in vivo* PET studies. A good regional correlation between radiotracer activity [carbonyl-¹¹C]WAY-100635 and 5-HT_{1A} receptor distribution was found except for small brain nuclei such as the raphe nucleus. In contrast, binding studies carried out in brain slices with the tritiated radioligand [³H]-OH-DPAT revealed substantial tracer uptake in small midbrain nuclei. Thus, the failure of PET to detect ligand binding in these areas could be attributed to partial-volume effects (Drevets et al., 1999; Kumar and Mann, 2007). Despite the absence of a CBV response in DR, we cannot exclude the possibility that the changes observed in 5-HT_{1A} terminal fields result at least in part, from activation of somatodentritic 5-HT_{1A} receptors in the raphe nucleus. Thus, 8-OH-DPAT at the dose used was reported to almost

completely silence DR neurons and affect the firing of cortical and hippocampal serotonergic neurons (Sprouse and Aghajanian, 1988; Hjorth and Sharp, 1991).

The decrease in CBV induced by 8-OH-DPAT could be antagonized by WAY-100635 in a rat study (Scanley et al., 2001), suggesting a selective effect of 8-OH-DPAT. In contrast, in our study using mice, the CBV response was not fully reversed by WAY-100635, although the antagonist was administered at a high dose. This discrepancy between species is currently not understood but may be due to a different ratio between the number of receptors, their respective affinity of the drug, and the concentration of each drug. Interestingly, a slight decrease in CBV, albeit smaller than that elicited by the agonist, was observed after administration of WAY-100635 alone. This suggests an intrinsic activity of WAY-100635, as previously reported (Scanley et al., 2001). However, other studies using electrophysiological recording *in vitro*, demonstrated that WAY-100635 blocks the effect of 5-HT agonists in a dose-dependent fashion at both, post-synaptic 5-HT_{1A} receptors in ascending 5-HT projection areas such as cingulate cortex, hippocampus, and amygdala, and somatodendritic 5-HT_{1A} receptors located on dorsal raphe nucleus 5-HT neurons, but cannot activate the 5-HT_{1A} receptor on its own. Studies in anesthetized animals further demonstrated that WAY-100635 does not increase extracellular 5-HT levels (Assié and Koek, 1996) or serotonergic firing in dorsal raphe nucleus (Fletcher et al., 1996). From these findings, we speculate that the weak CBV decrease elicited by WAY-100635 is independent of the 5-HT_{1A} receptor.

Our data showing no change in body temperature and tcpCO₂ values during and after drug injection indicate that hemodynamic parameters are not perturbed in our experimental set-up. Although 8-OH-DPAT was previously reported to affect body temperature in mice, through 5-HT_{1A} and 5-HT₇ receptor activation (Hedlund et al., 2004), this is unlikely to happen under our experimental conditions: artificial ventilation, moderate level of isoflurane and the use of an in-built warm water circulation were carefully adjusted to obtain stable physiological parameters and hemodynamic conditions through the fMRI experiment. Another potential confound to be considered is a drug-induced change in systemic blood pressure which might affect the CBV measurements. Several lines of evidence allow eliminating such a contribution. 1) Significant changes in BP would cause global and not region-specific changes in CBV following the injection of 8-OH-DPAT. 2) It was shown that 8-OH-DPAT produces a short-lasting increase in diastolic blood pressure (BP) in the rat at doses of 0.3 mg/kg and higher (Centurion et al., 2006) potentially via vascular α 1-adrenoceptors for which it possesses a moderate receptor affinity (pK_{0.1}: 5.3, Yoshio et al., 2001). At the doses used (0.02–0.1 mg/kg i.v.) 8-OH-DPAT is therefore unlikely to cause a significant BP response. 3) BP data obtained in the rat after i.v. infusion of 0.1 mg/kg 8-OH-DPAT (Scanley et al., 2001) showed a transient decrease in BP of short duration (1 min): neither the temporal pattern nor magnitude of BP change correlated with the temporal CBV profile assessed by fMRI. Clearly, an on-line measurement of BP during fMRI in mice would greatly help to exclude potential systemic effects. However, it remains highly demanding in anesthetized, ventilated and paralyzed mice and might—if carried out invasively—have its own confounding effect on the CNS challenge induced.

The absence of a CBV response in HOM 5-HT_{1A}-R knock-out mice clearly indicates the central role of this receptor system in mediating the 8-OH-DPAT induced hemodynamic changes. This is in line with autoradiography studies in knock-out mice showing total lack of specific binding of (³H) 8-OH-DPAT to 5-HT_{1A} receptors throughout the brain (Ramboz et al., 1998). The minor increase in CBV observed in HOM following drug administration might be due to drug interaction with other receptor systems, which may exhibit minor affinity for 8-OH-DPAT. 5-HT₇ receptors, located primarily in hippocampal and cortical areas, and in thalamic structures, can bind prototypical 5-HT_{1A} agonists with high affinity (Bard et al., 1993; Gustafson et al., 1996). Upon activation of this receptor system an increase in CBV is expected because the 5-HT₇ receptor has the opposite action on adenylate cyclase (AC) than the 5-HT_{1A} receptor; while the 5-HT_{1A} receptor is negatively coupled to AC, the 5-HT₇ receptor regulates it positively (Hannon and Hoyer, 2008). We therefore hypothesize that in HOM animals with a constitutively absent expression of the 5-HT_{1A} receptor the positive CBV response observed after administration of 0.1 mg/kg 8-OH-DPAT is mediated through activation of the 5-HT₇ receptor, as this is the now predominating activation profile. In HET and WT mice the CBV alteration potentially mediated through 5-HT₇ would remain masked by the pronounced CBV decrease upon the specific 5-HT_{1A} receptor activation. Further experiments are required to address this issue.

Mapping brain activity following non-invasive pharmacological stimulation in 5-HT_{1A} knock-out animals is of great interest because these mice have increased innate and conditioned anxiety-related behaviors (Parks et al., 1998; Ramboz et al., 1998; Gingrich and Hen, 2001; Gross et al., 2002). Since fMRI allows the analyses of large areas of the mouse brain, it is an attractive method for mapping potential changes in the underlying neuronal circuitry. The approach also lends itself to characterize a disease phenotype and evaluate the effects of therapeutic interventions by compounds such as selective serotonin reuptake inhibitors (SSRIs) or benzodiazepine inverse agonists targeting the 5-HT_{1B} or GABA_A receptor subunits, which have been reported to potentially contribute to the reported anxiety-related phenotype (Olivier et al., 2001; Pattij et al., 2002; Toth, 2003).

In summary, the present study demonstrates the feasibility of mapping region-specific and dose-dependent changes in serotonergic neurotransmission in the mouse by stimulation with the 5-HT_{1A} ligand 8-OH-DPAT using non-invasive fMRI. Resulting Δ CBV_% maps reflect the functional response to receptor activation, and provide complementary information to 5-HT_{1A} receptor imaging using PET. The specificity of the pharmacological paradigm was evaluated by comparing the hemodynamic response after co-injection of the 5-HT_{1A} agonist with the specific and potent 5-HT_{1A} receptor antagonist WAY-100635 and in 5-HT_{1A} knock-out mice. This fMRI protocol may be applied to investigate 5-HT_{1A} receptor functions in genetically engineered or experimental mouse models displaying a depression- and/or anxiety-related phenotype. It may also complement cognitive modulatory methods based on responses to cognitive challenge that have already been used in human (see review by Anderson et al., 2008).

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Contributors

TM and HR designed the study protocols and undertook the statistical analyses; FR conducted the functional MRI studies and the image analysis; HR and TBF planned breeding and conducted genotyping of the 5-HT_{1A} knock-out population; AB and CB contributed to the functional MRI studies and the image analysis; TM managed the literature searches and wrote the first draft of the manuscript. IMM and MR were involved in the design of the study and made a significant contribution to the writing of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

TM, FR, HR, AB, TBF, CB, IMM and MR have no disclosures or conflicts of interest to report.

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