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ORIGINAL INVESTIGATION

Pharmacological and behavioral characterization of the 5-HT_{2A} receptor in C57BL/6N mice

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Abstract
Rationale The serotonin (5-HT) 2A receptor is implicated in numerous psychiatric disorders, making it an important, clinically relevant target. Despite the availability of transgenic mouse lines, the native mouse 5-HT_{2A} receptor is not well-characterized.
Objectives The goals of the current study were to determine 5-HT_{2A} and 5-HT_{2C} receptor densities in mouse cortex, establish a pharmacological profile of the mouse 5-HT_{2A} receptor, and determine the effects of chronic drug treatment on 5-HT_{2A} receptor density and 5-HT_{2A} receptor-mediated behavior.
Methods Receptor densities were determined in cortex and frontal cortex via saturation binding assays using [^{3}H] ketanserin or [^{3}H]mesulergine. A pharmacological profile was established by displacing [^{3}H]ketanserin binding with several ligands. Chronic treatment with 5-$HT_{2A/2C}$ receptor agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI), 5-HT_{2A} receptor antagonist, MDL 11939, or vehicle was followed by 5-HT_{2A} receptor density determination. Head twitch responses (HTRs) were counted on select days.
Results Mice had high 5-HT_{2A}, but low 5-HT_{2C} receptor densities. Ligand binding affinities for mouse 5-HT_{2A} receptors correlated with rat, but not rabbit or human, affinities. Chronically DOI-treated mice displayed reduced HTRs and 5-HT_{2A} receptor density compared to saline-treated mice. Receptor density was unchanged following chronic treatment with MDL 11939.
Conclusions The current study provides some basic information about mouse 5-HT_{2A} and 5-HT_{2C} receptors and provides comparisons to rats, rabbits, and humans. The current chronic agonist treatment study demonstrated an important similarity between the 5-HT_{2A} receptor in mice, rats, and rabbits, while antagonist treatment revealed an interesting difference from previous studies in rabbits.

Keywords Mouse · 5-HT_{2A} receptor · 5-HT_{2C} receptor · Affinity · Receptor density · Cortex · Frontal cortex · Rat · Receptor regulation · Pharmacological profile

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Introduction

Serotonin (5-HT) 2A receptors are distributed throughout the brain in mammals, with highest receptor densities found in the cortex and frontal cortex (López-Giménez et al. 2002; Pazos et al. 1985, 1987; Pompeiano et al. 1994). The 5-HT_{2A} receptor is involved in hallucinogenic activity (Aghajanian and Marek 1999; González-Maeso et al. 2007) and plays a role in learning and memory (Harvey 2003). Additionally, the 5-HT_{2A} receptor is implicated in numerous psychiatric disorders, including schizophrenia (Aloyo et al. 2009; Richtand et al. 2008), depression (Berg et al. 2008; Marek et al. 2003; Michelsen et al. 2008), obsessive-compulsive disorder (El Mansari and Blier 2006; Marek et al. 2003; Steeves and Fox 2008), and autism (Marek et al. 2003). Given the potential as a therapeutic target, 5-HT_{2A} receptors have been studied in several species. In the mouse, however, receptor density of the 5-HT_{2A}—and the closely related 5-HT_{2C}—receptor, ligand binding affinities for the 5-HT_{2A} receptor, and biochemical and behavioral effects of chronic treatment with 5-HT_{2A} receptor agonists and antagonists are poorly characterized.

Among the few studies of 5-HT_{2A} and/or 5-HT_{2C} receptor densities in the mouse brain, reported B_{max} values

vary considerably across laboratories, ranging from approximately 20–400 fmol/mg protein in frontal cortex (Canal et al. 2010; Goodwin et al. 1984; Hayslett and Tizabi 2005; Heal et al. 1985; Rioux et al. 1999). In the aforementioned studies, when the 5-HT_{2C} receptor density was determined, it was not measured in the same region as the 5-HT_{2A} receptor. Thus, the current study began with determinations of 5-HT_{2A} and 5-HT_{2C} receptor densities in mouse cortex and frontal cortex.

The 5-HT_{2A} receptor sequence is highly conserved between species; the mouse 5-HT_{2A} receptor shares 97%, 89%, and 91% sequence identity with rat, rabbit, and human 5-HT_{2A} receptors, respectively (The Uniprot Consortium; Ensembl). Although 5-HT_{2A} receptor sequences are similar between many species, binding affinity studies found several ligands that bind differently to rat 5-HT_{2A} receptors compared to rabbits, pigs, monkeys, and humans (Aloyo and Harvey 2000; Johnson et al. 1994; Nelson et al. 1993). Moreover, several groups reported that mutations of a single amino acid in the 5-HT_{2A} receptor sequence can dramatically change its pharmacological profile (Kao et al. 1992; Johnson et al. 1994, 1997; Braden and Nichols 2007). Therefore, a pharmacological profile for the mouse 5-HT_{2A} receptor was developed in the current study. Several common and species-differentiating 5-HT_{2A} receptor ligands were used for this purpose, including ketanserin, mesulergine, MDL 11939, spiperone, LY 53857, ergonovine, lysergic acid diethylamide (LSD), and 2,5-dimethoxy-4-iodoamphetamine (DOI).

Few studies of chronic treatment with 5-HT_{2A} receptor antagonists or agonists have been performed using mice and it is not clear whether mouse 5-HT_{2A} receptors respond similarly to rats and/or rabbits following chronic treatment with 5-HT_{2A} receptor agonists and antagonists. Thus, mice in the current study were chronically treated with the 5-$HT_{2A/2C}$ receptor agonist, DOI, 5-HT_{2A} receptor antagonist, MDL 11939, or vehicle and cortical 5-HT_{2A} receptor density was determined following chronic drug treatment. In addition, head twitch responses (HTRs) were quantified on select days during treatment. The mouse head twitch response is a distinct, rapid, rotational movement of the head around the rostral–caudal axis. Similar to rat head shakes and rabbit head bobs, mouse HTRs are mediated by the 5-HT_{2A} receptor (Dursun and Handley 1996; González-Maeso et al. 2003; Rinaldi-Carmona et al. 1993a), with some modulatory influence exerted by 5-HT_{2C} receptors (Canal et al. 2010). Changes in the number of head movement behaviors elicited by 5-HT_{2A} receptor agonists correlate with changes in cortical 5-HT_{2A} receptor density in mice, rats, and rabbits following repeated drug administration (Blackshear and Sanders-Bush 1982; Dave et al. 2007; Goodwin et al. 1984; Heal et al. 1985; Metz and Heal 1986; Rinaldi-Carmona et al. 1993a, b). Measurement of HTRs on select days of an experiment functions as a simple, non-invasive, behavioral indicator of 5-HT_{2A} receptor activation and cortical receptor density.

Methods

Animals

Adult male C57BL/6N mice (20–30 g) were purchased from Harlan Labs and housed in groups of two to six per cage with free access to food and water. Adult male Sprague–Dawley rats (300–350 g) were housed individually with free access to food and water. Mice were sacrificed by cervical dislocation rapidly followed by decapitation. Rats were sacrificed by decapitation. Whole cerebral cortices or frontal cortices were removed, frozen on dry ice, and stored at −75°C until assayed. These studies were performed in accordance with the National Institutes of Health Guide "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985) and with our Institutional Animal Care and Use Committee approval.

Dissection

Dissection of whole and frontal cortex was performed over ice. First, the corpus callosum was severed and the left and right hemispheres were separated. For whole cortex, the hippocampus was then removed and cortex was dissected using microdissection forceps. Care was taken to ensure that no striatal or other subcortical tissue was included. Frontal cortex was defined as the region of cortex that is anterior to the anterior portion of the lateral ventricles and striatum.

Chemicals and reagents

[^{3}H]Ketanserin (67 Ci/mmol) was purchased from Perkin Elmer (Waltham, MA, USA). [^{3}H]Mesulergine (78 or 88 Ci/mmol) was purchased from Amersham/GE Life Sciences (Piscataway, NJ, USA) or American Radiolabeled Chemicals (St. Louis, MO, USA). Ergonovine maleate, DOI hydrochloride, LSD, LY 53857, prazosin hydrochloride, sodium hydroxide (NaOH), and bovine serum albumin were purchased from Sigma-Aldrich/RBI (St. Louis, MO, USA). Ketanserin hydrochloride, α-Phenyl-1-(2-phenylethyl)-4-piperidinemethanol (MDL 11939), spiperone hydrochloride, and 8-[5-(2,4-Dimethoxy-5-(4-trifluoromethylphenylsulphonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (RS 102221) were purchased from Tocris Bioscience (Ellisville, MO, USA). Bio-Rad protein assay dye reagent concentrate was purchased from Bio-Rad (Philadelphia, PA, USA).

Tissue preparation

On the day of the experiments, whole cortices from individual mice, pooled frontal cortices from two to three mice, or frontal cortices from individual rats were homogenized and prepared using methods described previously (Aloyo and Harvey 2000) prior to use in saturation binding or cold displacement assays. To summarize, tissue was homogenized in 50 mM Tris HCl buffer (1:10, *w/v*, pH 7.4, 4°C) and centrifuged. The supernatant was discarded, pellet resuspended in 50 mM Tris HCl buffer (1:50, *w/v*, pH 7.4, 4°C) and centrifuged. The second homogenization–centrifugation step was repeated. The supernatant was discarded and the pellet was resuspended (to a concentration of 8 mg tissue/ml tris) in 20 mM Tris HCl buffer (pH 7.4, 25°C) and homogenized. The tissue homogenate was then used in assays. Samples of tissue homogenate from each experiment were saved and stored at −75°C to later assay protein content.

5-HT_{2A} receptor analysis

Binding assays were performed at 25°C in 20 mM Tris HCl buffer (pH 7.4) and used 4 mg of cortical (or frontal cortical) homogenate per tube. [^{3}H]Ketanserin was used to analyze 5-HT_{2A} receptors. RS 102221 (30 nM, final concentration) and prazosin (30 nM, final concentration) were used to block [^{3}H]ketanserin binding to 5-HT_{2C} receptors and α_1 adrenergic receptors, respectively. Saturation binding studies were performed using six to eight concentrations of [^{3}H]ketanserin (0.04–4.3 nM, final concentration) in a 1 ml assay (final volume). A single concentration of [^{3}H]ketanserin (0.39–0.47 nM, final concentration) was used for cold displacement studies, which were performed in the presence of 5 mM Mg^{2+}, prazosin (30 nM, final concentration), and RS 102221 (30 nM, final concentration), and used six to 12 concentrations of unlabelled drug. Nonspecific binding was determined via addition of spiperone (100 nM, final concentration). Specific [^{3}H]ketanserin binding in buffer was used as a control for maximal binding in cold displacement experiments. Tubes from [^{3}H]ketanserin binding experiments were incubated for 2 h in a 25°C water bath, then rapidly filtered through Whatman GF/B filters (presoaked in 0.5% polyethylenimine) and washed three times with 3 ml of 20 mM Tris HCl buffer (pH 7.4, 4°C). Radioactivity on the filters was determined by liquid scintillation counting. In addition to mouse experiments, [^{3}H]ketanserin was used to determine the binding affinity of MDL 11939 at rat 5-HT_{2A} receptors. Cold displacement of a single concentration of [^{3}H]ketanserin (0.22–0.54 nM, final concentration) was performed using 11 concentrations of MDL 11939 and the same methods as described above for the mouse.

5-HT_{2C} receptor analysis

All binding assays were performed at 25°C in 20 mM Tris–HCl buffer (pH 7.4) and used 4 mg of mouse cortical (or frontal cortical) homogenate per tube. [^{3}H]Mesulergine was used to analyze 5-HT_{2C} receptors. Spiperone (30 nM, final concentration) was used to block [^{3}H]mesulergine binding to 5-HT_{2A} receptors. Saturation binding studies were performed using six to eight concentrations of [^{3}H]mesulergine (0.06–4.1 nM, final concentration) in a 1 ml assay (final volume). Tubes from these experiments were incubated for 2 h in a 25°C water bath, then rapidly filtered through Whatman GF/B filters (presoaked in 0.5% polyethylenimine) and washed three times with 3 ml of 20 mM Tris–HCl buffer (pH 7.4, 4°C). Radioactivity on the filters was determined by liquid scintillation counting.

Protein assay

Protein content of tissue homogenate samples was determined using the microassay procedure of the Bio-Rad Protein Assay, based on the method described by Bradford (1976). Protein-free "blank" tubes and tubes containing protein standards (ranging 2.5–20 μg/ml protein) were assayed in duplicate and average absorbance was plotted with final protein concentration. A linear regression was produced using the protein standard graph. Thawed tissue homogenate aliquots were assayed in triplicate and protein

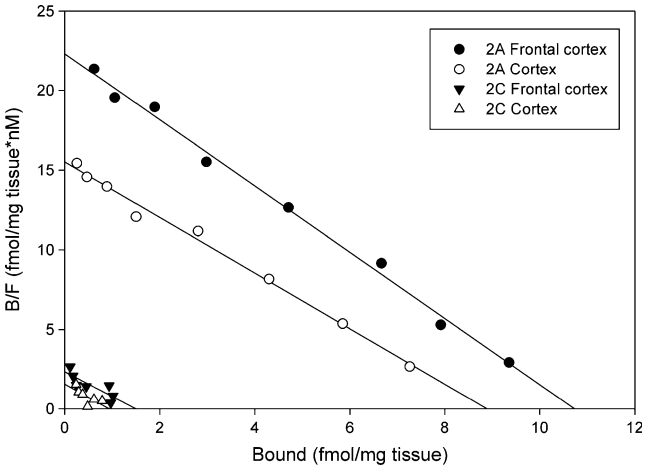

Fig. 1 Representative Scatchard plots produced from [^{3}H]ketanserin binding at the 5-HT_{2A} receptor and from [^{3}H]mesulergine binding at the 5-HT_{2C} receptor. [^{3}H]Ketanserin binding used whole cortices of individual mice (*empty circle*) or pooled mouse frontal cortices (*filled circle*). Experiments were repeated 16 times using whole cortex and 13 times using pooled frontal cortices. [^{3}H]Mesulergine binding used whole cortices of individual mice (*empty triangle*) or pooled mouse frontal cortices (*inverted filled triangle*). [^{3}H]Mesulergine binding experiments were repeated three times using each brain region

Table 1 Serotonin 2A and 2C receptor densities and binding affinities in mouse cortex and frontal cortex

	[^{3}H]Ketanserin (5-HT$_{2A}$)		[^{3}H]Mesulergine (5-HT$_{2C}$)	
	Cortex (95% CI)	Frontal cortex (95% CI)	Cortex (95% CI)	Frontal cortex (95% CI)
B_{max} (fmol/mg protein)	114.4 (91.3–137.4)	143.1 (107.6–178.7)	8.51 (−5.6–22.6)	13.4 (0.2–26.5)
K_d (nM)	0.53 (0.49–0.56)	0.48 (0.43–0.53)	0.44 (0.09–0.79)	0.61 (−0.15–1.37)
n	16	13	3	3

Mouse 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor B_{max} and K_d values were determined via saturation binding assays using [^{3}H]ketanserin or [^{3}H]mesulergine for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor binding, respectively. No significant differences were observed between mean B_{max} or K_d values in cortex or frontal cortex when using either [^{3}H]ketanserin or [^{3}H]mesulergine. Values given as mean with 95% CI

concentration in each sample was determined using the average absorbance of a sample and the equation for the protein standard regression.

Drug preparation

All drugs were prepared fresh each day for injection. DOI was dissolved in sterile saline and MDL 11939 was dissolved in glacial acetic acid and brought to pH 6.5 with NaOH. Drug solutions were prepared at concentrations necessary to inject 5 μl of solution per gram of mouse.

MDL 11939 pretreatment and acute DOI administration

Mice were injected (i.p.) with 2.95 mg/kg MDL 11939 or vehicle and returned to their home cages. Thirty minutes after pretreatment, 1 mg/kg DOI or saline was injected (i.p.) and behavior was recorded as described below.

Chronic administration of DOI

Mice were injected (i.p.) once daily with 1 mg/kg DOI or saline for 8 days. Twenty-four hours after the eighth injection, all mice were challenged with 0.75 mg/kg DOI, their behavior recorded as described below, and were sacrificed. The dose used for chronic DOI treatment was chosen based on a dose shown to alter frontocortical 5-HT$_{2A}$ receptor density in rats and rabbits following chronic treatment (Aloyo et al. 2001; Anji et al. 2000; Hensler and Truett 1998; McKenna and Peroutka 1989; Smith et al. 1999). The dose used for DOI challenge was chosen based on a dose shown to elicit a significant, but submaximal, increase in HTRs (Weiss et al. 2003) to avoid a ceiling effect.

Chronic administration of MDL 11939

Mice were injected (i.p.) once daily with 2.95 mg/kg MDL 11939 or vehicle for 4 or 8 days. Twenty-four hours after the final treatment, all mice were challenged with 0.75 mg/kg DOI. Mouse behavior was recorded as described below, and the mice were sacrificed. The dose of MDL 11939 used was chosen based on a dose previously shown to alter frontocortical 5-HT$_{2A}$ receptor density in rabbits following chronic treatment (Dave et al. 2007).

Behavior

On select days, individual mice were placed in an empty, transparent, plastic 7.5″×11.75″ cage (Allentown Caging,

Table 2 Ligand binding affinities at mouse cortical 5-HT$_{2A}$ receptors

Antagonists	K_i (nM)	95% CI	Agonists	K_i (nM)	95% CI
Ketanserin	0.53	0.49–0.56	Ergonovine	22.1	6.32–37.9
Mesulergine	2.43	−0.08 to 4.94	LSD	0.80	−0.39 to 2.00
MDL 11939	2.25	1.40–3.10	DOI (high)	6.52	1.62–11.4
Spiperone	0.40	0.24–0.57	DOI (low)	92.8	32.5–153
LY 53857	4.18	1.94–6.41			

K_i values for mouse cortical 5-HT$_{2A}$ receptors were determined for the ligands at the 5-HT$_{2A}$ receptor. All ligands examined, except DOI, displaced [^{3}H]ketanserin at a single class of binding sites. DOI displaced [^{3}H]ketanserin at two classes of binding sites. LSD, ketanserin, and spiperone had very high affinities for the mouse 5-HT$_{2A}$ receptor. MDL 11939, LY 53857, mesulergine, and DOI (at its high affinity site) had moderate affinity for the mouse 5-HT$_{2A}$ receptor. Ergonovine and the low affinity binding site for DOI had the lowest affinities for the mouse 5-HT$_{2A}$ receptor of the ligands tested. Values given as mean with 95% CI of three to four separate experiments

Inc., Allentown, NJ, USA) for 20 min immediately following injection and their behavior was recorded using a JVC Everio (GZ-MG21u) camcorder. Mice treated with MDL 11939 or vehicle for 4 days had behavior recorded on days 1 and 4. Mice treated with MDL 11939, DOI, or vehicle for 8 days had behavior recorded on days 1, 2, 4, and 8. Mice challenged with DOI 24 h after treatment also had their behavior recorded. Mice pretreated with MDL 11939 or vehicle prior to acute DOI or vehicle administration were recorded for 20 min following DOI or vehicle administration. Videos were watched by at least one blinded observer and HTRs that were not contiguous with other behaviors were counted.

Literature survey

Values for 5-$HT_{2A/2C}$ receptor density in rabbit, rat, and human frontal cortices were obtained from the literature. K_i or IC_{50} values for ketanserin, mesulergine, MDL 11939, spiperone, LY 53857, ergonovine, DOI, and LSD were obtained from the literature; IC_{50} values were converted to K_i using the Cheng-Prusoff (1973) equation.

Data analysis

Binding data were analyzed using EBDA/LIGAND (McPherson 1985), a nonlinear curve-fitting program. K_d, B_{max}, and K_i values were determined from [^{3}H]ketanserin or [^{3}H]mesulergine saturation binding assays and displacement of [^{3}H]ketanserin by unlabelled ligands. Statistical analysis was performed using Systat 7.0 software (SPSS, Chicago, IL, USA). Two-sample *t* tests were performed to identify differences between means of B_{max}, K_d, and K_i values from receptor analysis and chronic drug administration experiments. Two-sample *t* tests were also performed to identify differences between mean HTRs of treatment groups upon DOI challenge. A mixed analysis of variance (ANOVA) was used to determine if there was a significant ($p<0.05$) main effect or interaction of treatment and days of treatment on HTRs during chronic drug administration experiments. When a significant interaction of treatment and days of treatment were found, univariate *F* tests were performed to determine on which days there was a significant difference between treatment groups. One-way ANOVA was used to analyze the HTRs from MDL 11939- or vehicle-pretreated, DOI- or saline-treated mice. A significant effect was followed up by a pairwise comparison with a Bonferroni adjustment. Pearson product moment correlation was used to determine correlation between mouse and other species binding affinity values. Data from the current study are presented as mean ± 95% confidence interval (CI) unless otherwise noted.

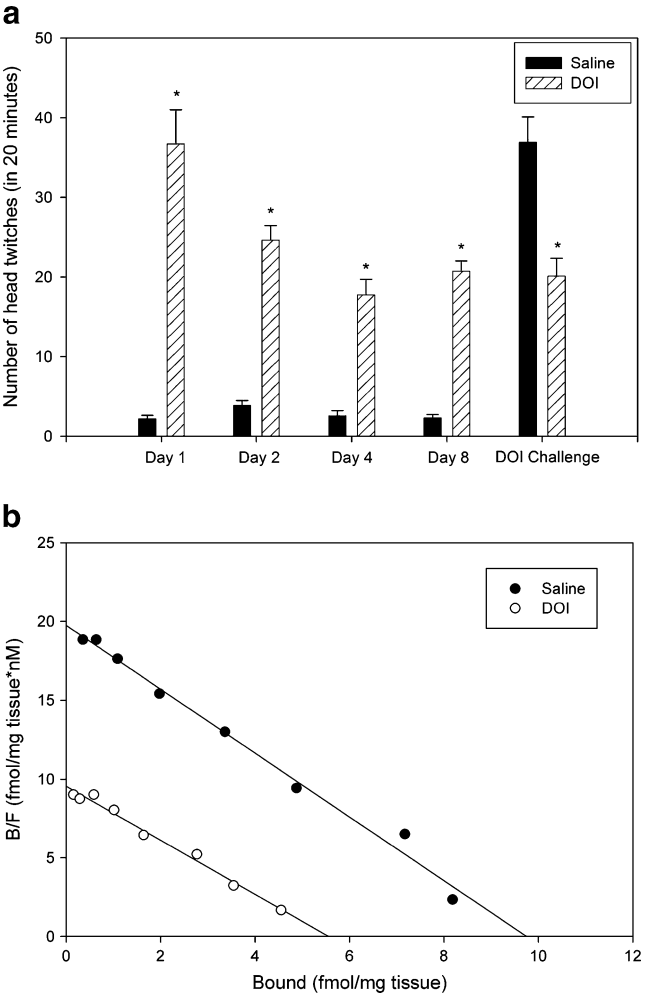

Fig. 2 **a** Head twitch responses in mice treated once daily for 8 days with 1 mg/kg DOI or saline. The number of head twitches occurring immediately after DOI or saline administration is shown for days 1, 2, 4, 8. Additionally, on day 9, both DOI- and saline-treated mice were challenged with 0.75 mg/kg DOI and the number of head twitches elicited was observed. Tolerance to the behavioral effects of DOI was observed as early as the second day of treatment, when DOI-elicited HTRs were decreased to two thirds the value on day 1. Following the 0.75 mg/kg DOI challenge, mice that had been chronically treated with saline exhibited significantly more HTRs than mice chronically treated with DOI. Values given as mean ± SEM from 10 or 11 mice for saline and DOI treatment groups, respectively. *$p<0.001$ compared to saline group HTRs. **b** Representative Scatchard plot from DOI- and saline-treated mice in 8-day DOI experiment. Mice were treated once daily with 1 mg/kg DOI (*empty circle*) or saline (*filled circle*) for 8 days, received an injection with 0.75 mg/kg DOI 24 h following the final treatment, and were sacrificed. Whole cortices from each mouse were removed and saturation binding was performed using six to eight concentrations of [^{3}H]ketanserin. Cortical 5-HT_{2A} receptor density was significantly greater in saline- than DOI-treated mice. Data are from single experiments and are representative of the results from nine experiments performed for the saline treatment group and ten experiments performed for the DOI-treatment group

Results

Serotonin 2A receptor density in mouse cortex and frontal cortex

Serotonin 2A receptor densities in mouse cortex and frontal cortex were determined via saturation binding of [^{3}H] ketanserin. Scatchard analysis of binding results showed that [^{3}H]ketanserin bound to a single class of binding sites in mouse cortex and frontal cortex (Fig. 1). In whole cortex, mean B_{max} and K_i values for [^{3}H]ketanserin (5-HT$_{2A}$ receptor) binding from 16 separate assays were 114.4 fmol/mg protein (95% CI, 91.3–137.4) and 0.53 nM (95% CI, 0.49–0.56), respectively. In pooled frontal cortex, mean B_{max} and K_i values for [^{3}H]ketanserin binding from 13 separate assays were 143.1 fmol/mg protein (95% CI, 107.6–178.7) and 0.48 nM (95% CI, 0.43–0.53), respectively (Table 1). No significant difference was found between whole cortex and frontal cortex B_{max} or K_i values (t test; p=0.141 and p=0.093, respectively).

Serotonin 2C receptor density in mouse cortex and frontal cortex

Serotonin 2C receptor densities in mouse cortex and frontal cortex were determined via saturation binding of [^{3}H] mesulergine. Scatchard analysis of binding results showed that [^{3}H]mesulergine bound to a single class of binding sites in mouse cortex and frontal cortex (Fig. 1). In whole cortex, mean B_{max} and K_i values for [^{3}H]mesulergine (5-HT$_{2C}$ receptor) binding from three separate assays were 8.51 fmol/mg protein (95% CI, −5.6–22.6) and 0.44 nM (95% CI, 0.09–0.79), respectively. In pooled frontal cortex, mean B_{max} and K_i values for [^{3}H]mesulergine from three separate assays were 13.4 fmol/mg protein (95% CI, 0.2–26.5) and 0.61 nM (95% CI, −0.15–1.37), respectively (Table 1). No significant difference was found between whole cortex and frontal cortex B_{max} or K_i values (t test; p=0.341 and p=0.425, respectively).

Serotonin 2A receptor ligand binding affinities in mouse

Binding affinities of several commonly-used and species-differentiating 5-HT$_{2A}$ receptor antagonists (mesulergine, MDL 11939, spiperone, LY 53857) and agonists (ergonovine, DOI, LSD) of the 5-HT$_{2A}$ were determined via dose-dependent displacement of [^{3}H]ketanserin in mouse cortex. Nonlinear curve fitting analyses of three to four separate assays revealed that seven of the eight 5-HT$_{2A}$ receptor ligands examined inhibited [^{3}H]ketanserin binding at a single class of sites. The eighth ligand, DOI, inhibited [^{3}H]ketanserin binding at two classes of sites. Most K_i values obtained, ranging from 0.4 nM for spiperone to 6.52 nM for the high affinity DOI binding site, indicate that these ligands bind with high affinity for the 5-HT$_{2A}$ receptor (Table 2). Ergonovine and the low affinity DOI binding site were exceptions to these findings, with K_i values of 22.1 and 92.8 nM, respectively.

MDL 11939 pretreatment blocks DOI-elicited HTRs

Mice were pretreated with vehicle or 2.95 mg/kg MDL 11939 30 min prior to administration of saline or 1 mg/kg DOI to confirm that a 2.95 mg/kg MDL 11939 dose is behaviorally active and capable of blocking DOI-elicited HTRs. One-way analysis of variance revealed a significant main effect of treatment ($F_{3,20}$=43.59, p<0.001). Post hoc pairwise comparisons with a Bonferroni adjustment revealed that vehicle-pretreated, DOI-treated mice exhibited significantly more head twitches than either saline-treated group (p<0.001) or the MDL 11939-pretreated, DOI-treated group (p<0.001). No other significant differences between groups were found.

DOI HTRs and 5-HT$_{2A}$ receptor binding

A significant interaction between treatment and days of treatment were found via mixed ANOVA ($F_{3,57}$=9.65, p<0.001). The simple main effect of treatment on days of treatment was determined via post hoc univariate F tests,

Table 3 Cortical 5-HT$_{2A}$ receptor density and K_d following repeated DOI or MDL 11939 treatment

	B_{max} (fmol/mg protein)			K_d (nM)		
	Vehicle	Drug	Change (%)	Vehicle	Drug	Change (%)
DOI (95% CI)	98.7 (61.1–136.2)	58.1* (35.9–80.3)	−41	0.54 (0.50–0.58)	0.74 (0.50–0.98)	+37
MDL 11939 (8 days; 95% CI)	96.8 (63.0–130.5)	85.5 (49.9–121.0)	−12	0.58 (0.45–0.70)	0.56 (0.48–0.63)	−3
MDL 11939 (4 days; 95% CI)	132.5 (103.6–161.5)	130.5 (84.9–176.1)	−1.5	0.51 (0.41–0.60)	0.58 (0.44–0.72)	+16

Mice received daily doses of 1 mg/kg DOI or vehicle for 8 days or daily doses of 2.95 mg/kg MDL 11939 or vehicle for 4 or 8 days. Values given as mean with 95% CI from six mice for MDL 11939 experiments and nine to ten mice for DOI experiments

*p<0.05 compared to vehicle

which revealed that DOI-treated mice exhibited significantly more HTRs than vehicle-treated mice on all days of behavioral observation ($p<0.001$; $F_{1,19}=58.63$, $F_{1,19}=107.1$, $F_{1,19}=49.47$, and $F_{1,19}=177.0$ for days 1, 2, 4, and 8, respectively; Fig. 2a). However, mice showed tolerance to the behavioral effects of DOI, beginning 24 h after the first dose and persisting for the duration of the experiment. DOI-treated mice exhibited significantly fewer HTRs than vehicle-treated mice when challenged with 0.75 mg/kg DOI 24 h after their final 1 mg/kg DOI or vehicle injection (t test; $p<0.001$). Consistent with behavioral observations, cortical 5-HT$_{2A}$ receptor density in DOI-treated mice was significantly decreased compared to vehicle-treated mice after chronic treatment (t test; $p=0.043$; Fig. 2b; Table 3).

MDL 11939 HTRs and 5-HT$_{2A}$ receptor binding

MDL 11939-treated mice exhibited fewer HTRs than vehicle-treated mice on all days observed (days 1, 2, 4, and 8 of 8-day treatment; Fig. 3a; days 1 and 4 of 4-day treatment; data not shown). Additionally, a mixed ANOVA of HTRs during 8-day treatment revealed significant main effects of treatment ($F_{1,11}=37.42$, $p<0.001$) and days of treatment ($F_{3,33}=3.23$, $p=0.035$), but no significant interaction ($F_{3,33}=1.99$, $p=0.134$). Analysis of HTRs during the 4-day experiment revealed a significant interaction between treatment and day of treatment ($F_{1,10}=5.49$, $p=0.041$). The simple main effect of treatment on days of treatment was determined via post hoc univariate F tests, which revealed that MDL 11939-treated mice exhibited significantly fewer HTRs than vehicle-treated mice on all days of behavioral observation ($p<0.01$; $F_{1,10}=14.71$ and $F_{1,10}=16.89$ for days 1 and 4, respectively). When challenged with 0.75 mg/kg DOI 24 h after their final MDL 11939 or vehicle injection, however, no difference in HTRs was found between treatment groups (t test; $p=0.188$ and $p=0.923$ for 8- and 4-day treatments, respectively). Consistent with behavioral observations, cortical 5-HT$_{2A}$ receptor density in MDL 11939-treated mice was not significantly different from vehicle-treated mice (t test; $p=0.577$ and $p=0.923$ for 8- and 4-day treatments, respectively; Fig. 3b). Binding data from chronic DOI and MDL 11939 treatments is summarized in Table 3.

Comparisons with other species

Receptor density data for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors from rats, rabbits, and humans were compiled for comparison with mice (Table 4). Table 5 compares ligand-binding affinities at the mouse 5-HT$_{2A}$ receptor with literature values for rats, rabbits, and humans. No K_i value for MDL 11939 in rat frontal cortex is present in the literature and was therefore determined. Binding affinity data are represented graphically in Fig. 4 (excluding low affinity DOI K_i values). Studies of

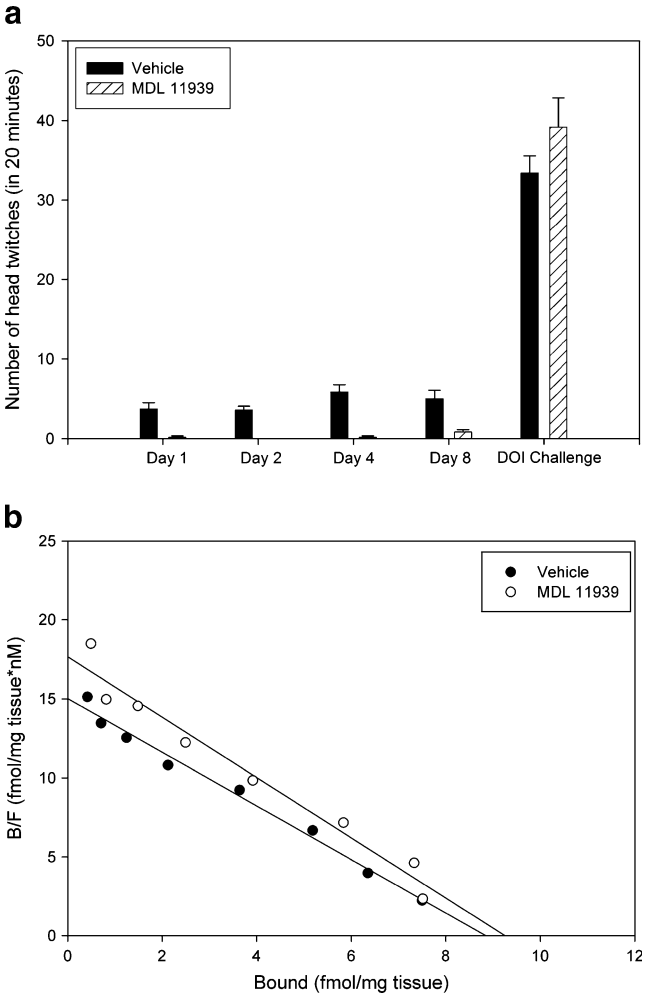

Fig. 3 **a** Head twitch responses in mice treated once daily for 8 days with 2.95 mg/kg MDL 11939 or saline. The number of head twitches occurring immediately after MDL 11939 or saline administration is shown for days 1, 2, 4, 8. Additionally, on day 9, both MDL 11939- and saline-treated mice were challenged with 0.75 mg/kg DOI and the number of head twitches observed. A significant main effect of treatment was found over the course of MDL 11939 and saline treatment ($p<0.001$). No significant difference was observed between treatment groups when all mice received a 0.75 mg/kg DOI challenge 24 h after their final treatment. Values given as mean ± SEM from seven or six mice for vehicle and MDL 11939 groups, respectively. **b** Representative Scatchard plots from MDL 11939- and vehicle-treated mice in 8-day MDL 11939 experiment. Mice were treated once daily with 2.95 mg/kg MDL 11939 (*empty circle*) or vehicle (*filled circle*) for 8 days, received an injection with 0.75 mg/kg DOI 24 h following the final treatment, and were sacrificed. Whole cortices from each mouse were removed and saturation binding was performed using six to eight concentrations of [^{3}H]ketanserin. Cortical 5-HT$_{2A}$ receptor density did not differ significantly between vehicle- and MDL 11939-treated mice. Data are from single experiments and are representative of the results from seven experiments performed for the vehicle treatment group and six experiments performed for the MDL 11939-treatment group

rats treated chronically with DOI and rabbits treated chronically with DOI or MDL 11939 have been published. Table 6 summarizes the effects of chronic DOI or MDL 11939 treatments on 5-HT$_{2A}$ receptor density in mice, rats,

Table 4 Serotonin 2A and 2C receptor densities in mouse, rat, rabbit, and humans

	B_{max} (fmol/mg protein)		2A:2C ratio	% total 5-$HT_{2A/2C}$ receptor density	
	5-HT_{2A} receptor	5-HT_{2C} receptor		5-HT_{2A}	5-HT_{2C}
Mouse CTX (95% CI)	114.4[d] (91.3–137.4)	8.51[e] (−5.6–22.6)	13.44	93.1	6.9
Mouse FCTX (95% CI)	143.1[f] (107.6–178.7)	13.4[e] (0.2–26.5)	10.68	91.4	8.6
Rat[a]	232.8	43.6	5.34	84.2	15.8
Rabbit[b]	102.7[g]	42.2[g]	2.43	70.9	29.1
Human[c]	314	162.75	1.93	65.9	34.1

Mouse 5-HT_{2A} and 5-HT_{2C} receptor densities were determined by saturation binding using [^{3}H]ketanserin and [^{3}H]mesulergine, respectively

[a] Fone et al. 1998; Hoyer et al. 1985, 1986; Hulihan-Giblin et al. 1994; Leonhardt et al. 1992; Pranzatelli and Balletti 1992

[b] Schindler et al., unpublished

[c] Hoyer et al. 1985; Leonhardt et al. 1992; Pazos et al. 1985; Pranzatelli and Balletti 1992

[d] Mean from 16 separate determinations

[e] Mean from three separate determinations

[f] Mean from 13 separate determinations

[g] Mean from 18 separate determinations

and rabbits. In all three species, animals treated chronically with DOI had decreased 5-HT_{2A} receptors in the cortex or frontal cortex. Unlike that obtained in rabbits, mouse cortical 5-HT_{2A} receptor density did not change following 4 or 8 days of 2.95 mg/kg MDL 11939 treatments. No studies in which rats were treated with MDL 11939 and 5-HT_{2A} receptor density was examined could be found in the literature.

Discussion

The current study was designed to provide a more complete pharmacological profile of the mouse 5-HT_{2A} receptor than is presently available. Additionally, the relationship between 5-HT_{2A} and the closely related 5-HT_{2C} receptor densities in cortex and frontal cortex was examined. Whole

Table 5 K_i values for several ligands at mouse, rat, rabbit, and human cortical 5-HT_{2A} receptors

	Mouse[a]	Rat[b]	Rabbit[c]	Human[d]
Ketanserin	0.53 (0.49–0.56)	1.82	0.54±0.10	2.13[e,f]
Mesulergine	2.43 (−0.08–4.94)	7.23[e]	7.25	68.37[e]
MDL 11939	2.25 (1.40–3.10)	1.85[a] (0.74–2.96)	0.54±0.06	6.06[g]
Spiperone	0.40 (0.24–0.57)	0.81	0.19±0.02	1.26[e]
LY 53857	4.18 (1.94–6.41)	11.24	3.91±0.63	17.9
Ergonovine	22.1 (6.32–37.9)	27.42	0.32±0.02	2.06
LSD	0.80 (−0.39–2.00)	3.63	0.46±0.045	2.79
DOI (high)	6.52 (1.62–11.4)	18.9	0.49±0.36	6
DOI (low)	92.8 (32.5–153)	nd	17.0±1.6	nd

nd Not determined

[a] K_i or K_d values were determined as described in the methods section and presented as the mean with the 95% CI. All other data were obtained from the literature and are presented as the mean ± SEM where multiple literature values were available

[b] Battaglia et al. 1983, 1984; Bonhaus et al. 1995; Hagen et al. 1994; Johnson et al. 1996; Leonhardt et al. 1992; Leysen et al. 1982, 1988; Lyon et al. 1987; Meltzer et al. 1989; Neale et al. 1987; Nelson et al. 1993; Pazos et al. 1984; Rinaldi-Carmona et al. 1992; Roth et al. 1992; Sadzot et al. 1989; Schotte et al. 1983; Schreiber et al. 1995; Wainscott et al. 1996

[c] Aloyo and Harvey 2000, Schindler et al., unpublished; Weber et al. 1997

[d] Bonhaus et al. 1995; Hagen et al. 1994; López-Giménez et al. 1998; Nelson et al. 1993; Pazos et al. 1984; Sadzot et al. 1989; Schotte et al. 1983

[e] Includes K_i values obtained using [^{3}H]MDL 100907

[f] Includes K_d values obtained using [^{3}H]Ketanserin

[g] Includes values obtained using [^{125}I]DOI

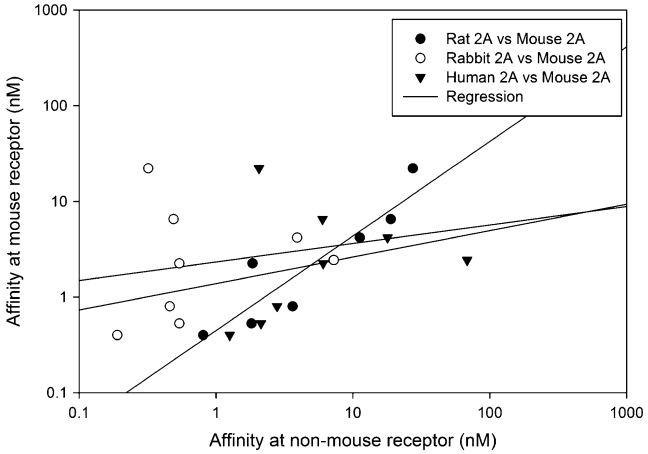

Fig. 4 The correlation between ligand affinities for the mouse 5-HT_{2A} receptor and their affinities for the rat (*filled circle*), rabbit (*empty circle*), or human (*inverted filled triangle*) 5-HT_{2A} receptors. Affinities of ligands, also presented in Table 5, were determined by displacing specifically bound [^{3}H]ketanserin from mouse, rat, rabbit, or human 5-HT_{2A} receptors. A significant correlation (r=0.913, p<0.001) between ligand affinities at mouse and rat receptors was found. Correlations between ligand affinities for mouse and rabbit 5-HT_{2A} receptors or mouse and human receptors were not significant (rabbit, r=−0.162, p>0.05; human, r=−0.154, p>0.05)

mouse cortex and frontal cortex both exhibited high levels of 5-HT_{2A} receptors. These results suggest a widespread distribution of 5-HT_{2A} receptors throughout all regions of mouse cortex. Consistent with saturation binding results of the current study, autoradiography in mouse brain revealed high levels of 5-$HT_{2A/2C}$ agonist, [^{125}I]DOI, and selective 5-HT_{2A} antagonist, [^{3}H]MDL 100907, binding throughout the cortex of 129S6/SvEv (González-Maeso et al. 2007) and C57BL/6J mice (López-Giménez et al. 2002), respectively. In addition to similarities to autoradiography results, the number of DOI-elicited HTRs in C57BL/6N mice in the current study is similar to previous reports using C57BL/6J mice (Weiss et al. 2003). Thus, it is unlikely that the receptor densities currently determined using C57BL/6N mice are markedly different from other mouse strains.

Unlike the mouse 5-HT_{2A} receptor, 5-HT_{2C} receptor density was very low in both mouse cortex and frontal cortex. Mouse 5-HT_{2C} receptor density was observed to be quite different compared to rats, rabbits, and humans (Table 4). In both regions, mouse 5-HT_{2C} receptors account for less than 10% of the combined 5-$HT_{2A/2C}$ receptor density. In contrast, 5-HT_{2C} receptors account for 15.8%, 29.1%, and 34.1% of the combined 5-$HT_{2A/2C}$ receptor densities in rat, rabbit, and human cortex, respectively. Future studies will be needed to confirm the current results and elucidate any behavioral or biochemical differences in species with higher or lower cortical 5-HT_{2C} receptor densities.

The current study established mouse 5-HT_{2A} receptor binding affinities of several common 5-HT_{2A} receptor ligands and allowed a preliminary interspecies comparison of ligand binding at the 5-HT_{2A} receptor. Interestingly, the mouse 5-HT_{2A} receptor binding affinities obtained were significantly correlated with affinities of the rat but not the human or rabbit receptors. The correlation between mouse and rat ligand affinities is consistent with an early characterization of the 5-HT_{2A} receptor performed by Peroutka et al. (1981). Peroutka et al. found a strong correlation between drug potencies to inhibit HTRs in mice and inhibit [^{3}H] spiroperidol binding in rat cortex. Differences in ligand binding affinities between mouse and rabbit or human receptors are probably attributable to species differences in 5-HT_{2A} receptor sequences, as single amino acid changes in the 5-HT_{2A} receptor sequence are capable of causing dramatic changes to its ligand binding profile (Braden and Nichols 2007; Johnson et al. 1994, 1997; Kao et al. 1992).

Consistent with previous reports, acute treatment with DOI in the present study elicited significantly more HTRs compared to vehicle treatment (Darmani et al. 1990; Dursun and Handley 1996; Fox et al. 2009; Rinaldi-Carmona et al. 1993a; Weiss et al. 2003). Mice treated once daily with DOI for 8 days, however, rapidly developed tolerance to the behavioral effects. DOI-elicited HTRs were reduced by 33% on the second day of treatment. This tolerance to DOI-elicited HTRs was similar to that observed by Darmani et al. (1990), who chronically treated male ICR mice with 2.5 mg/kg DOI. Tolerance to the behavioral effects of DOI was maintained throughout the 8 days in the current experiments. Furthermore, mice treated with 1 mg/kg DOI for 8 days exhibited tolerance to a smaller dose of DOI (0.75 mg/kg) administered on the ninth day. These results are in marked contrast to the results obtained by Darmani et al. (1990). They observed tolerance to DOI-elicited HTRs through the fifth day of chronic DOI treatment; HTRs counted on days six and eight were not significantly different from HTRs on day 1 (Darmani et al. 1990). Future studies are necessary to determine if differences in mouse strains and DOI doses used account for these inconsistencies.

Table 6 Summary of changes in cortical 5-HT_{2A} receptor density following DOI or MDL 11939 treatment in the mouse, rat, and rabbit

Drug	Dose (mg/kg)	Duration (days)	Mouse	Rat	Rabbit
DOI	1	8	↓	↓[a]	↓[b]
MDL 11939	2.95	4 or 8	↔	nd	↑[c]

nd Not determined

[a] Anji et al. 2000; Chaouloff et al. 1993; Chaouloff et al. 1997; Hensler and Truett 1998; McKenna and Peroutka 1989; Smith et al. 1999

[b] Aloyo et al. 2001

[c] Dave et al. 2007

The current study is the first to determine that cortical 5-HT_{2A} receptor density in mice is significantly decreased following chronic administration of DOI. Mice, similar to rats and rabbits, displayed decreased 5-HT_{2A} receptor density (Table 6). Thus, chronic administration of DOI decreases both 5-HT_{2A} receptor density and DOI-elicited HTRs. The positive correlation between drug-induced head twitch behavior and cortical receptor density observed in this study is consistent with other studies in mice (Table 7). Similar correlations between 5-HT_{2A}-mediated head movements and 5-HT_{2A} receptor density have also been demonstrated in rats and rabbits (Aloyo et al. 2001; Dave et al. 2007; Leysen et al. 1989; Leysen and Pauwels 1990). These results suggest that certain head movement behaviors, such as mouse head twitches, might be used as behavioral indicators of changes in cortical 5-HT_{2A} receptor density.

In the present study, acute administration of the selective 5-HT_{2A} antagonist, MDL 11939, reduced spontaneous and DOI-elicited head twitches. Dursun and Handley (1996) also reported that spontaneous and DOI-elicited HTRs can be blocked with 5-HT_{2A} receptor antagonists. These results support the conclusion that activation of 5-HT_{2A} receptors mediates head twitches, whether spontaneous or DOI-induced. Similar to acute treatment, chronic treatment with MDL 11939 reduced spontaneous HTRs in mice on all days observed. Mice chronically treated with MDL 11939, however, did not differ from vehicle-treated mice in either DOI-elicited HTRs or 5-HT_{2A} receptor densities. A greater dose may be necessary to see a change in receptor density in mice, but pilot studies using higher doses of MDL 11939 revealed dose-dependent sedative-like effects and a dramatic reduction in most behaviors (data not shown), therefore making treatment with a larger dose of MDL 11939 unsuitable for the current study.

Numerous studies, primarily using rats, have demonstrated that a "paradoxical" downregulation in response to chronic treatment with 5-HT_{2A} receptor antagonists (for reviews see Gray and Roth 2001; van Oekelen et al. 2003). However, two antagonists have resulted in the up-regulation of 5-HT_{2A} receptors. Chronic administration of SR 46349B, for example, up-regulates 5-HT_{2A} receptor density in rats, mice, and rabbits (Rinaldi-Carmona et al. 1993a,b; Scarlota et al. unpublished). Similarly, repeated administration of MDL 11939 to rabbits leads to an increase in 5-HT_{2A} receptor density (Dave et al. 2007). Surprisingly, in the current study, repeated administration of a behaviorally active dose of MDL 11939 neither up- nor downregulated 5-HT_{2A} receptor density. This lack of effect suggests that the mechanisms regulating 5-HT_{2A} receptor density may be different in different species and that "paradoxical" regulation may not be a universal property of 5-HT_{2A} receptors. Based on the current results, future

Table 7 Effects of chronic drug treatment on mouse 5-HT_{2A} receptor density in frontal cortex and drug-induced head twitches

Class	Treatment	HTRs	5-HT_{2A} receptor density	Dose (mg/kg)	Duration (days)	Drug used to elicit HTRs
MAOI	Tranylcypromine	↓[a]	↓[a]	5.6	35	5-MeODMT
SSRI	Zimeldine	↓[a]	↓[a]	20	14	5-MeODMT
TCA	Mianserin	↓[a]	↓[a]	2.1	14	5-HTP
		↓[b]	↓[b]	20	14	5-MeODMT
	Desmethylimipramine	↓[a]	↓[a]	27	14	5-MeODMT
		↓[d]	↓[d]	5	3.5[g]	5-MeODMT
2A/2 C inverse agonist	SR 46349B	↑[c]	↑[c]	5	3.5[g]	5-HTP
		↑[c]	↑[c]	10	3.5[g]	5-HTP
Other	Electroconvulsive shock	↑[a]	↑[a]	n/a	10[h]	5-HTP
		↑[d]	↑[d]		3	5-MeODMT
	5,7-DHT	↑[e]	↑[e]	0.05[f]	1	5-MeODMT

5-MeODMT, 5-methoxy-dimethyltryptamine; 5-HTP, 5-hydroxytryptophan

[a] Goodwin et al. 1984

[b] Blackshear and Sanders-Bush 1982

[c] Rinaldi-Carmona et al. 1993a

[d] Metz and Heal 1986

[e] Heal et al. 1985; HTRs counted 2 weeks after 5,7-DHT injection, 5-HT_{2A} receptor density determined 3 weeks after 5,7-DHT injection

[f] 0.05 mg Intracerebroventricular injection

[g] Drug administered twice per day for 3 days, once on the fourth day

[h] Five electroconvulsive shock sessions over 10 days

investigations using a variety of 5-HT_{2A} receptor ligands in mice and other species are warranted.

Interestingly, the same dose and durations of MDL 11939 treatment that had no effect on mouse cortical 5-HT_{2A} receptor density increased rabbit cortical 5-HT_{2A} receptor density (Dave et al. 2007). This finding highlights the difficulty of predicting antagonist effects on 5-HT_{2A} receptor regulation; downregulation of 5-HT_{2A} receptors after chronic antagonist treatment is variable between ligands or, at least in the current study, species. At the time of this writing, no studies using rats for a similar experiment have been published. A study using rats could help determine if the lack of change after 4 or 8 days of MDL 11939 treatment was common among rodents or limited to mice. Although further study is necessary, the difference between the current results using mice and previous studies in rabbits suggests the presence of interspecies differences in 5-HT_{2A} receptor regulation by certain compounds.

Mice, like humans, have high cortical 5-HT_{2A} receptor density and are therefore useful for studying cortical 5-HT_{2A} receptor regulation and function. However, in contrast to humans, rats, and rabbits, mouse cortical 5-HT_{2C} receptor density was very low, especially relative to 5-HT_{2A} receptor density. Assuming 5-HT_{2C} receptors are pharmacologically and biochemically similar across species, their low density in mice relative to 5-HT_{2A} receptors could result in preferential binding at cortical 5-HT_{2A} receptors over 5-HT_{2C} receptors. Additionally, differences in cortical 5-HT_{2A} to 5-HT_{2C} receptor density ratios combined with differences in ligand binding affinities at 5-HT_{2A} receptors may limit cross-species translation of 5-$HT_{2A/2C}$ receptor ligand effects in cortex. Despite these differences, 5-HT_{2A} receptor density decreased similarly in mouse, rat, and rabbit following chronic treatment with DOI. Chronic treatment with MDL 11939, however, revealed a differential effect on 5-HT_{2A} receptor density in mice and in rabbits. The current study presents a more complete picture of the mouse 5-HT_{2A} receptor than previously existed. Future studies can establish additional similarities and differences between mouse and rat, rabbit, and human 5-HT_{2A} and 5-HT_{2C} receptors, and should help to further refine the appropriate use of mice to elucidate the role of 5-HT_{2A} receptors in learning, memory, and psychiatric disorders.

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