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The Native Serotonin 5-HT$_{5A}$ Receptor: Electrophysiological Characterization in Rodent Cortex and 5-HT$_{1A}$-Mediated Compensatory Plasticity in the Knock-Out Mouse

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Abstract

The 5-HT$_{5A}$ receptor is the least understood serotonin (5-HT) receptor. Here, we electrophysiologically identify and characterize a native 5-HT$_{5A}$ receptor current in acute *ex vivo* brain slices of adult rodent prefrontal cortex. In the presence of antagonists for the previously characterized 5-HT$_{1A}$ and 5-HT$_2$ receptors, a proportion of layer V pyramidal neurons continue to show 5-HT-elicited outward currents in both rats and mice. These 5-HT currents are suppressed by the selective 5-HT$_{5A}$ antagonist, SB-699551, and are not observed in 5-HT$_{5A}$ receptor knock-out mice. Further characterization reveals that the 5-HT$_{5A}$ current is activated by submicromolar concentrations of 5-HT, is inwardly rectifying with a reversal potential near the equilibrium potential for K^+ ions, and is suppressed by blockers of Kir3 channels. Finally, we observe that genetic deletion of the inhibitory 5-HT$_{5A}$ receptor results in an unexpected, large increase in the inhibitory 5-HT$_{1A}$ receptor currents. The presence of functional prefrontal 5-HT$_{5A}$ receptors in normal rodents along with compensatory plasticity in 5-HT$_{5A}$ receptor knock-out mice testifies to the significance of this receptor in the healthy prefrontal cortex.

Introduction

Serotonin (5-HT) receptors control a number of physiological processes, most notably emotional behaviors. The 5-HT$_{5A}$ receptor subtype is the least understood, despite its widespread expression in the human and rodent brains (Pasqualetti et al., 1998; Kinsey et al., 2001). To date, there has been limited functional evidence of the 5-HT$_{5A}$ receptor in the brain (Sprouse et al., 2004; Thomas et al., 2006), and its endogenous channel effector(s) remain uncertain (Grailhe et al., 2001; Noda et al., 2003). Given this lack of functional characterization in the native brain tissue, the 5-HT$_{5A}$ receptor remains only provisionally classified within the 5-HT receptor family (IUPHAR database) (Hannon and Hoyer, 2008).

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The recent development of the selective 5-HT_{5A} antagonist (SB-699551) (Corbett et al., 2005) and the generation of 5-HT_{5A} knock-out mice (Grailhe et al., 1999) have now made it possible to examine functional 5-HT_{5A} receptors within native *ex vivo* brain tissue. Here, for the first time, we identify and characterize functional 5-HT_{5A} receptor currents in cortical neurons and investigate the consequence of genetic deletion of the 5-HT_{5A} receptor on postsynaptic serotonin receptor signaling.

Materials and Methods

Experimental animals

Sprague Dawley rats, Sv129 mice, and C57BL/6 mice were obtained from Charles River. Serotonin 5-HT_{5A} receptor (*htr5A*) transgenic mice on an Sv129 background (Grailhe et al., 1999) were bred at the University of Toronto. We used male adolescent and adult rats [postnatal day (P) 46 ± 3; $n = 22$ rats] and adult mice (Sv129: P110 ± 7; $n = 45$ mice; C57BL/6: P223 ± 41; $n = 3$ mice).

Genotyping

To genotype sibling 5-$HT_{5A}^{+/+}$ and 5-$HT_{5A}^{-/-}$ mice for our experiments, the following PCR protocol was used: 95°C for 3 min, 35 cycles of (94°C for 45 s, 52°C for 45 s, and 72°C for 1 min), and 72°C for 10 min. The following primers were added to the PCR to amplify the 5-HT_{5A} wild-type allele: forward primer 5′-TTTCTAGCTGCGGCCACATTCACT-3′ and reverse primer 5′-TCATCACATTGGAGACACGCTT GC-3′. The following primers were added to the PCR to amplify the 5-HT_{5A} knock-out allele: forward primer 5′-ATTCGGCTATGACTGGGCACAACA-3′ and reverse primer 5′-GTAAAGCACGAGGAGGAAGC GGTCAGC-3′. The expected sizes of the PCR products were 340 bp and 676 bp for the wild-type and knock-out alleles, respectively.

Brain slice preparation

In brief, coronal slices (400 μm thick) were made from prefrontal cortex (4.20–2.52 mm from bregma for rats; 2.46–1.34 mm for mice). Excised brains were rapidly cooled with 4°C oxygenated sucrose ACSF (254 mM sucrose was substituted for NaCl), cut on a Dosaka Linear Slicer (SciMedia) and transferred to 30°C oxygenated ACSF (128 mM NaCl, 10 mM D-glucose, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgSO_4$, 3 mM KCl, 1.25 mM NaH_2PO_4; pH 7.4). Slices were allowed to recover for at least 1–2 h, then were placed in a superfusion chamber on the stage of a BX50WI microscope (Olympus). Regular bubbled ACSF (95% oxygen and 5% carbon dioxide; 31–33°C) flowed at a rate of 3–4 ml/min.

Electrophysiology

Whole-cell recording electrodes (3–4 MΩ) containing 120 mM potassium gluconate, 5 mM KCl, 2 mM $MgCl_2$, 4 mM K_2-ATP, 0.4 mM Na_2-GTP, 10 mM Na_2-phosphocreatine, and 10 mM HEPES buffer (adjusted to pH 7.3 with KOH) were used to patch layer V pyramidal neurons in medial prefrontal cortex under visual control. Currents were recorded using a Multiclamp 700b under continuous single-electrode voltage-clamp mode at a holding potential of −75 mV (Molecular Devices). Current–voltage (IV) relationships were obtained using 15 mV/s voltage ramps from −120 to −60 mV. The IV curve obtained at baseline was

subtracted from the IV curve obtained during 5-HT agonist application. All data were acquired at 20 kHz (reduced to 1 kHz for illustrations) and low-pass filtered at 3 kHz, using pClamp10.2/Digidata1440 software (Molecular Devices).

Rat layer V neurons (n = 149) had a resting potential of −80.5 ± 0.4 mV, spike amplitude of 87.2 ±0.5 mV, and input resistance of 92.1 ±3.0 MΩ. For mouse neurons, there were no significant differences in the neuronal properties by breeding location or *htr5A* genotype. Combined, Sv129 layer V neurons (n = 329) had a resting potential of −86.4 ± 0.4 mV, spike amplitude of 84.6 ± 0.3 mV, and input resistance of 170.5 ± 3.4 MΩ. Combined, Sv129 layer II/III neurons (n = 55) had a resting potential of −92.2 ± 0.9 mV, spike amplitude of 83.9 ± 0.9 mV, and input resistance of 144.3 ± 8.6 MΩ.

A current step (500 ms) twice the amplitude of the rheobase current was used to elicit a spike train. The firing frequency (f) of the first (f1), second (f2), and last (fL) interspike intervals were then used to calculate the burst index (f1/f2), adaptation index (fL/f2), and maximum frequency (Otsuka and Kawaguchi, 2008). Layer V neurons from5-$HT_{5A}^{+/+}$ and 5-$HT_{5A}^{-/-}$ mice displayed no difference in the bursting index (p = 0.9), maximum spike frequency (p = 0.9), adaptation index (p = 0.2), or mean interspike interval (p = 0.7). Moreover, the proportions of slow-adapting, slow-adapting with an initial doublet, and fast-adapting neurons did not differ between the genotypes (p = 0.3).

Pharmacology

Serotonergic currents were probed by adding serotonin (5-HT; 30 s) to the bath after a baseline period. Other drugs were also added to the bath: 2 μM tetrodotoxin (TTX), 3 μM baclofen, 1 mM barium chloride ($BaCl_2$), 10–300 nM WAY-100635, 10 μM caboxamindotryptamine maleate (5-CT), 1–2 μM ketanserin, 10 μM SB-699551, 10 μM (R)-(+)-hydroxy-DPAT hydrobromide (8-OH-DPAT). All compounds were obtained from Sigma, Tocris Bioscience, or Alomone and stored in stock solutions at −20°C.

Western blot

Prefrontal cortical brain tissue was collected from 5-$HT_{5A}^{+/+}$ and 5-$HT_{5A}^{-/-}$ mice (n = 6 per genotype), as described above. Medial sections were dissected and processed to extract total protein (Millipore). Equal amounts of denatured protein extracts (20 μg) were separated by SDS-PAGE on 12% gels and transferred to nitrocellulose membranes. Membranes were incubated overnight at 4°C with an anti-5-HT_{1A} receptor polyclonal primary antibody (1:4000, AB15350; Millipore) (Jacobsen et al., 2011), incubated for 1 h with a peroxidase-conjugated secondary antibody (1:7000; Jackson Immunoresearch), and visualized using chemiluminescence. Band intensities were quantified using ImageJ and normalized to β-actin.

Statistical analysis

The peak amplitude of the serotonergic current was measured using Clampfit software (Molecular Devices). This measurement was obtained by subtracting the 1 s averaged holding current at the peak of the 5-HT response from holding current at the baseline. Statistical comparisons for within-cell responses to either one or several pharmacological

agents were determined using Student's two-tailed paired *t* tests or repeated-measures ANOVA, respectively. To evaluate between-cell responses, we used Student's two-tailed unpaired *t* tests. We used Fisher's exact tests to compare the differences in proportions of neurons displaying a response of interest. IV curves were statistically analyzed using a comparison of fits between a straight line and a second-order polynomial. Data are expressed as mean ±SE and statistical comparisons evaluated at a significance level of 0.05.

Results

Evidence that the 5-HT$_{5A}$ receptor mediates an unidentified 5-HT current in cortex

The 5-HT$_{5A}$ receptor is found in the rodent cerebral cortex (Grailhe et al., 1999; Kinsey et al., 2001) and expressed preferentially in layer V neurons (Belgard et al., 2011), together with the more extensively studied 5-HT$_{1A}$ and 5-HT$_2$ receptors. To examine the 5-HT$_{5A}$ receptor current, the latter receptors were blocked with 10–30 nM WAY-100635 and 1–2 μM ketanserin; higher concentrations were used for rapid blockade (10 min), followed by continued application of the lower concentrations. These concentrations were selected based on previous studies (Béïque et al., 2004; Goodfellow et al., 2009).

In the presence of these antagonists, we found that 5-HT (10 μM; 30 s) continued to elicit unidentified outward currents, which exceeded 3 times root mean square baseline noise and persisted for at least 60 s. These currents were observed in a proportion of layer V pyramidal neurons in the prefrontal cortex of Sprague Dawley rats (14/42 neurons; 33%; 24.8 ± 1.4 pA; Fig. 1*A*), Sv129 mice (42/114 neurons; 37%; 15.4 ±0.5 pA), as well as C57BL/6 mice (5/10 neurons; 50%; 14.8 ± 1.8 pA). These findings are consistent with previous reports of unidentified inhibitory effects of 5-HT in the rodent cortex (Amargós-Bosch et al., 2004; Villalobos et al., 2005; Zhong and Yan, 2011). Subsequent within-cell experiments showed that the unidentified 5-HT currents were resistant to TTX (2 μM, 20 min; rat, n =4; mouse, n = 5; Fig. 1*B,D*) and to antagonists of the glutamate and GABA receptors (100.2 ± 13.7% of baseline unidentified 5-HT current, n = 6, p = 0.9; Fig. 2*A*). In contrast, they were significantly suppressed by the 5-HT$_{5A}$ receptor antagonist, SB-699551 (10 μM, 20 min; Fig. 1*B,D*). These findings suggest the presence of functional 5-HT$_{5A}$ receptors in layer V neurons of the prefrontal cortex.

Control experiments using pharmacological tools and 5-HT$_{5A}^{-/-}$ transgenic mice

Since the prefrontal cortex also expresses receptors from the inhibitory 5-HT$_1$ receptor subfamily (Bruinvels et al., 1994; Amargós-Bosch et al., 2004), we performed a series of additional control experiments. First, we investigated whether the unidentified 5-HT current resulted from an incomplete blockade of the 5-HT$_{1A}$ receptor. The unidentified 5-HT current was not elicited by the 5-HT$_{1A}$ agonist, 8-OH-DPAT (10 μM; 5 min; rats, n = 5; mice, n = 7; Fig. 2*B*) and persisted following bath application of a higher concentration of the 5-HT$_{1A}$ antagonist, WAY-100635 (300 nM; 104 ±10.2% of baseline unidentified 5-HT current, n = 6, p = 0.6). Moreover, we did not observe an unidentified 5-HT current in layer II/III neurons (0 of 15 neurons; p = 0.003), cells with functional 5-HT$_{1A}$ receptors (Goodfellow et al., 2009) that do not express 5-HT$_{5A}$ receptors (Belgard et al., 2011). Second, additional experiments in layer V revealed that the unidentified 5-HT current was not blocked by the

selective 5-HT_{1B} antagonist, SB-224289 (2 μM; 10 min; 106.3 ± 10.8% of baseline unidentified current, $n = 5$, $p = 0.7$) and could not be elicited by the potent 5-$HT_{1E/1F}$ agonist, BRL54443 (1 μM, 3 min; $n = 4$). Finally, we found that the unidentified 5-HT current could, however, be mimicked by 5-CT (10 μM, 30 s; rats, $n = 6$; mice, $n = 17$; Fig. 2*B*), a mixed 5-HT receptor agonist with high affinity for the 5-HT_{5A} receptor (Matthes et al., 1993). Together, these findings suggest that the unidentified 5-HT current is not mediated by a member of the 5-HT_1 receptor family and further support the involvement of the 5-HT_{5A} receptor.

To test the hypothesis that the 5-HT_{5A} receptor mediates the unidentified 5-HT current, we recorded from mice with the deletion of the *htr5A* gene (5-$HT_{5A}^{-/-}$) and their littermate wild-type siblings (5-$HT_{5A}^{+/+}$) (Grailhe et al., 1999). As illustrated in Figure 2*C*, a substantial proportion of layer V neurons in 5-$HT_{5A}^{+/+}$ mice display unidentified 5-HT currents in the presence of the 5-HT_{1A} and 5-HT_2 receptor antagonists (17/36 neurons, 47%; 16.8 ± 1.6 pA). In contrast, recordings made in layer V of 5-$HT_{5A}^{-/-}$ mice under identical conditions did not reveal an unidentified 5-HT current (0/17 neurons, $p = 0.0003$). Examination of the spike firing patterns in 5-$HT_{5A}^{+/+}$ and 5-$HT_{5A}^{-/-}$ mice suggests that similar populations of neurons were recorded in both genotypes (see Materials and Methods, above).

Characterization of native 5-HT_{5A} receptor currents in adult prefrontal cortex

Next, we characterized the 5-HT_{5A} receptor currents in normal rodents. The 5-HT_{5A} current had a compelling influence on the excitability of pyramidal neurons in mice (Fig. 3*A*). When layer V neurons were injected with positive current to induce sustained action potential firing (2.5 ± 0.3 Hz, $n = 6$), stimulation of the 5-HT_{5A} current eliminated their firing (0 ± 0 Hz, $n = 6$, $p = 0.001$). This inhibitory influence of the 5-HT_{5A} current on neuronal excitability is likely enhanced by its reduction of the input resistance in layer V neurons (−30.4 ± 8.8 MΩ from baseline; $n = 9$; $p = 0.009$). Concentration-response analyses revealed that the 5-HT_{5A} receptor is activated by submicromolar levels of 5-HT (rat EC_{50}: 0.6 μM, 95% CI: 0.3–1.2 μM, $n = 5$; mouse EC_{50}: 0.9 μM, 95% CI: 0.4–1.9 μM, $n = 5$; Fig. 3*B*). Current–voltage analysis showed that the 5-HT_{5A} current is inwardly rectifying (4 of 4 neurons; comparison of fits, $p < 0.0001$) with a reversal potential (−98 mV, 95% CI: −98 to −99 mV) near the calculated equilibrium potential for K^+ ions (Fig. 3*C*). Extending this finding, the 5-HT_{5A} current can be suppressed by blockers of G-protein-linked inwardly rectifying K^+ (Kir3) channels: Ba^{2+} ions (1 mM, 10 min; $n = 5$) and Tertiapin-Q (0.1 μM, 20–40 min; $n = 5$; Fig. 3*D*). Together, these results demonstrate that in *ex vivo* brain slice, the 5-HT_{5A} receptor has relatively high affinity for 5-HT and elicits a K^+ current through activation of Kir3 channels.

To examine whether 5-HT_{5A} currents are enriched in a particular population of layer V neurons, we compared the spike firing characteristics (Otsuka and Kawaguchi, 2008) between wild-type mouse neurons with and without a 5-HT_{5A} current response. For this analysis, we used the wild-type 5-$HT_{5A}^{+/+}$ neurons from Figures 1*B1* and 2*C*, for which we had assessed the spiking pattern in response to an injection of twice the rheobase current ($n = 144$ neurons). We found that an unexpectedly high proportion of initial-doublet neurons

(burst index > 2.7) had 5-HT_{5A} current responses (19 of 26 neurons; 73%; $p = 0.0001$). Since neurons with similar firing patterns tend to project to the same brain region (Hattox and Nelson, 2007), 5-HT_{5A} receptors may suppress preferentially a specific type of prefrontal cortical output mediated by this class of neuron.

Genetic deletion of the 5-HT_{5A} receptor increases 5-HT_{1A} receptor currents

Since 5-HT modulates prefrontal cortex through several 5-HT receptors (Amargós-Bosch et al., 2004; Béïque et al., 2004), we investigated whether genetic deletion of *htr5A* gene altered the overall neuronal response to 5-HT. Recording in the absence of antagonists, we observed that the loss of the inhibitory 5-HT_{5A} receptor paradoxically increased 5-HT-elicited inhibitory outward currents in layer V neurons (5-$HT_{5A}^{+/+}$ neurons, $n = 36$; 5-$HT_{5A}^{-/-}$ neurons, $n = 35$; $p = 0.0003$; Fig. 4*A*). This supra-compensatory plasticity in 5-$HT_{5A}^{-/-}$ mice appeared to be mediated by an increase in 5-HT_{1A} receptor currents (baseline 5-HT current: 51.0 ±7.5 pA; after 30 nM WAY-100635: −1.8 ±1.8 pA; $n = 6$; $p = 0.002$; Fig. 3*B*). Interestingly, we detected no difference in medial prefrontal 5-HT_{1A} receptor protein content between 5-$HT_{5A}^{+/+}$ (0.52 ± 0.03 arbitrary units, $n = 6$) and 5-$HT_{5A}^{-/-}$ mice (0.47 ± 0.02 arbitrary units, $n = 6$; $p = 0.2$; Fig. 4*C*). To test the specificity of the electrophysiological effect for the 5-HT_{1A} receptor, we examined the magnitude of another $G_{\alpha i/o}$-mediated current using a selective $GABA_B$ agonist (baclofen; 3 μM, 30 s). In contrast, the $GABA_B$ outward currents were similar in 5-$HT_{5A}^{+/+}$ (67.4 ±3.7 pA, $n = 20$) and 5-$HT_{5A}^{-/-}$ mice (73.6 ± 4.8 pA, $n = 20$; $p = 0.3$). Next, we examined whether the increased 5-HT_{1A} receptor currents in 5-$HT_{5A}^{-/-}$ mice were restricted to the cortical layer with functional 5-HT_{5A} receptors (see Results, above). To this end, we examined the 5-HT_{1A}-mediated outward currents in layer II/III neurons in the absence of any antagonists (5-$HT_{5A}^{+/+}$ neurons, $n = 20$; 5-$HT_{5A}^{-/-}$ neurons, $n = 20$). A two-way ANOVA revealed a significant interaction between *htr5A* genotype and the prefrontal cortical layer (Fig. 4*A*). Together, these experiments suggest that genetic deletion of the 5-HT_{5A} receptor triggers a specific upregulation of 5-HT_{1A} outward currents selectively in layer V output neurons of the prefrontal cortex.

Discussion

In the present study, we provide direct evidence of functional, native 5-HT_{5A} receptors in cortical neurons of both rats and mice. We find that these receptors produce a small, inwardly rectifying K^+ current through Kir3 channels in a subpopulation of neurons, and this 5-HT current is absent in the cortex of 5-HT_{5A} receptor knock-out mice. Finally, we show that loss of the *htr5A* gene is sufficient to trigger the upregulation of another inhibitory 5-HT current mediated by the 5-HT_{1A} receptor. These results, to our knowledge, are the first to characterize functionally the 5-HT_{5A} receptor in *ex vivo* cortical brain tissue and to establish a previously unknown interaction between the 5-HT_{5A} receptor and the therapeutically relevant 5-HT_{1A} receptor.

Serotonergic inhibition of the prefrontal cortex is important for coordinating emotional behaviors (Puig and Gulledge, 2011). To date, this inhibition has been attributed entirely to 5-HT_{1A} receptors, despite evidence suggesting the presence of an additional, unidentified, inhibitory 5-HT effect (Amargós-Bosch et al., 2004, Villalobos et al., 2005; Zhong and Yan,

2011). Specifically, prefrontal 5-HT_{1A} receptors are thought to regulate emotional responses by inhibiting the major output neurons of the prefrontal cortex. Our findings, however, demonstrate a previously unappreciated role of 5-HT_{5A} receptors in modulating prefrontal neurons. Notably, the 5-HT_{5A} receptor and 5-HT_{1A} receptor display similar coupling to effectors (for 5-HT_{1A}, see Raymond et al., 1999; for 5-HT_{5A}, see Grailhe et al., 2001; present study) and efficacy for the 5-HT ligand (for 5-HT_{1A}, see Okuhara and Beck, 1998; for 5-HT_{5A}, see present study). Moreover, like the 5-HT_{1A} receptor, the 5-HT_{5A} receptor is expressed in a number of limbic regions, including the hippocampus and cortex (Grailhe et al., 1999; Kinsey et al., 2001). Despite these similarities, the 5-HT_{5A} knock-out mice do not display the anxiety phenotype observed in 5-HT_{1A} knock-out mice (Ramboz et al., 1998), but rather exhibit altered LSD-mediated explorative behaviors (Grailhe et al., 1999). It is tempting to speculate that the presence of the closely related 5-HT_{1A} and 5-HT_{5A} receptors in the same neuronal cells may serve as a biological safeguard, such that disruption of one receptor may induce compensatory up-regulation of the other receptor (Gingrich and Hen, 2000). In support of this hypothesis, we show that loss of *htr5A* gene strongly upregulates 5-HT_{1A} receptor-mediated currents in the prefrontal cortex. This interaction may have clinical implications since 5-HT_{1A} receptor agonists have been used in the treatment of mood disorders (Blier and Ward, 2003). Development of selective 5-HT_{5A} ligands is critical to improving our understanding the physiological relevance of this relatively unknown 5-HT receptor as well as elucidating its interactions with other members of the 5-HT receptor family.

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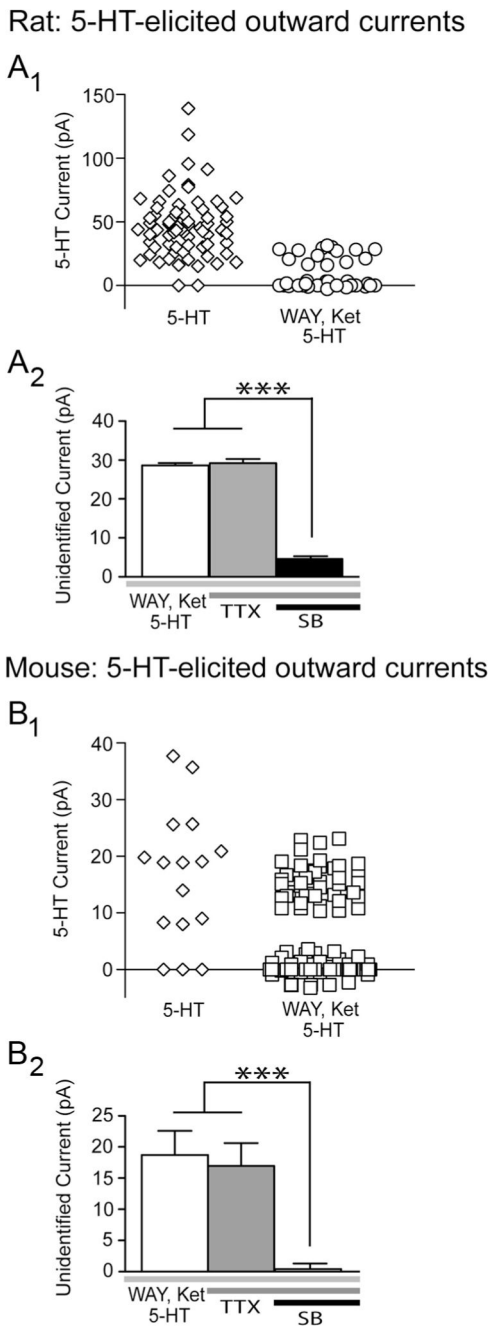

Figure 1.

An unidentified 5-HT current in prefrontal cortex of rat and mouse: evidence of functional 5-HT_{5A} receptors. In the rat (***A1***) and mouse (***B1***) prefrontal cortex, bath application of 5-HT (10 μM ; 30 s) elicits an unidentified outward current in the presence of the 5-HT_{1A} and 5-HT_2 antagonists, WAY-100635 (WAY) and ketanserin (Ket). The bar graphs summarize within-cell paired experiments from rats (***A2***) and mice (***B2***), showing that the baseline unidentified 5-HT current is a postsynaptic current that does not change in the presence of

TTX but is significantly suppressed by the 5-HT_{5A} antagonist, SB-699551 (SB) (repeated-measures ANOVA; rat, ***p =0.0001; mouse, ***p <0.0001).

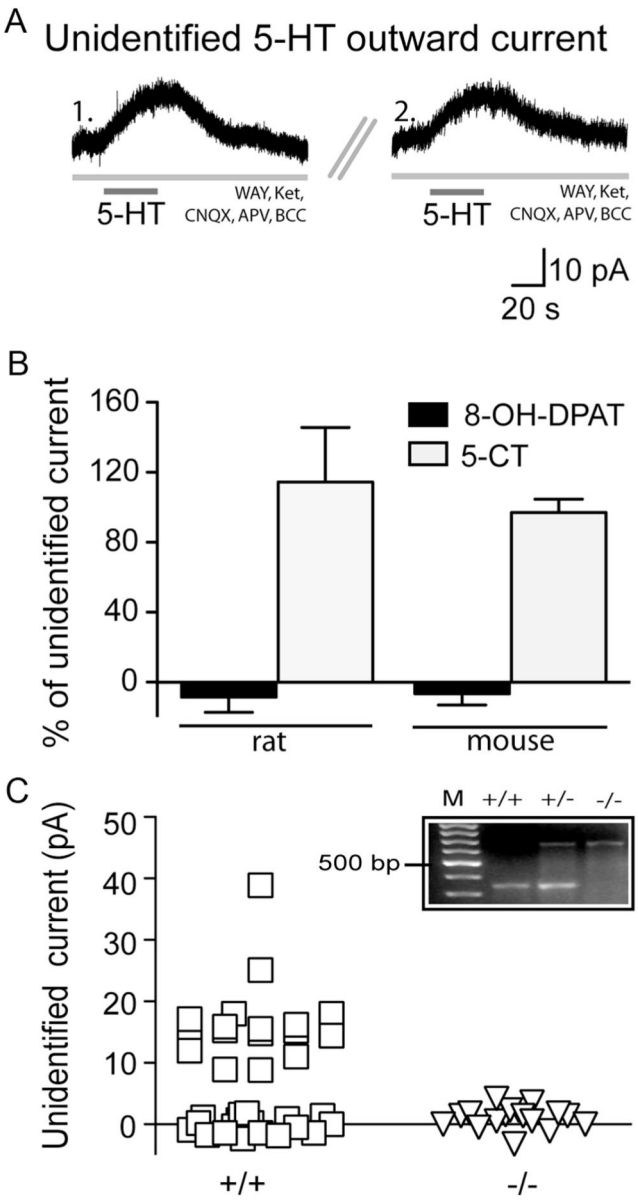

Figure 2.

Pharmacological and transgenic confirmation that the 5-HT_{5A} receptor mediates the unidentified 5-HT current. ***A***, Voltage-clamp traces showing the unidentified 5-HT current in the presence of WAY-100635 (WAY) and ketanserin (Ket) (1.) can be re-elicited upon repeat application of 5-HT following sufficient washout (5 min) (2.). ***B***, Under these conditions, the unidentified 5-HT outward current was not elicited by the 5-HT_{1A} agonist, 8-OH-DPAT (paired *t* test; rat, $p = 0.0007$; mice, $p < 0.0001$), but was elicited by the mixed 5-HT receptor agonist, 5-CT (paired *t* test; rat, $p = 0.7$; mice, $p = 0.4$). ***C***, In 5-$HT_{5A}^{+/+}$ mice, a substantial proportion of layer V neurons display an unidentified 5-HT current (squares). In sibling 5-$HT_{5A}^{-/-}$ mice, however, layer V neurons do not display this current (triangles; Fisher's exact test, $p = 0.0003$). Inset, PCR products derived from 5-HT_{5A} wild-type (+/+), heterozygous

knock-out (+/−), and homozygous knock-out (−/−) mice. Lane M corresponds to a 100 bp DNA ladder with the 500 bp marker labeled.

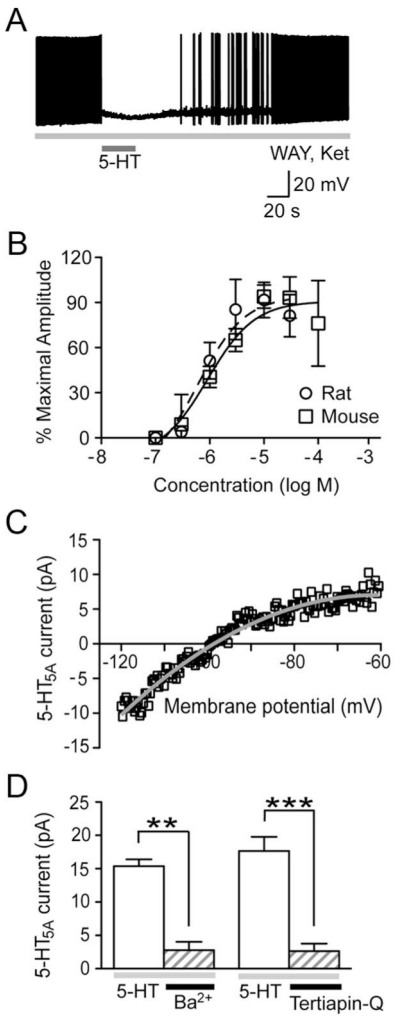

Figure 3.

Characterization of the 5-HT_{5A} current in the normal adult rodent cortex. ***A***, Current-clamp trace illustrates that the 5-HT_{5A} current can inhibit neuronal excitability resulting from a constant depolarizing current. ***B***, The concentration–response curves demonstrate that the 5-HT_{5A} receptor has relatively high affinity for applied 5-HT in both the rat and mouse prefrontal cortex. ***C***, Current–voltage graph illustrates that the 5-HT_{5A} response is inwardly rectifying and reverses near the calculated equilibrium potential for K^+ ions. ***D***, Bar graph shows that the 5-HT_{5A} current is suppressed by the Kir3 channel blockers: Ba^{2+} ions (paired *t* test; $^{**}p = 0.002$) and Tertiapin-Q (paired *t* test; $^{***}p = 0.001$). WAY: WAY-100635; Ket: ketanserin.

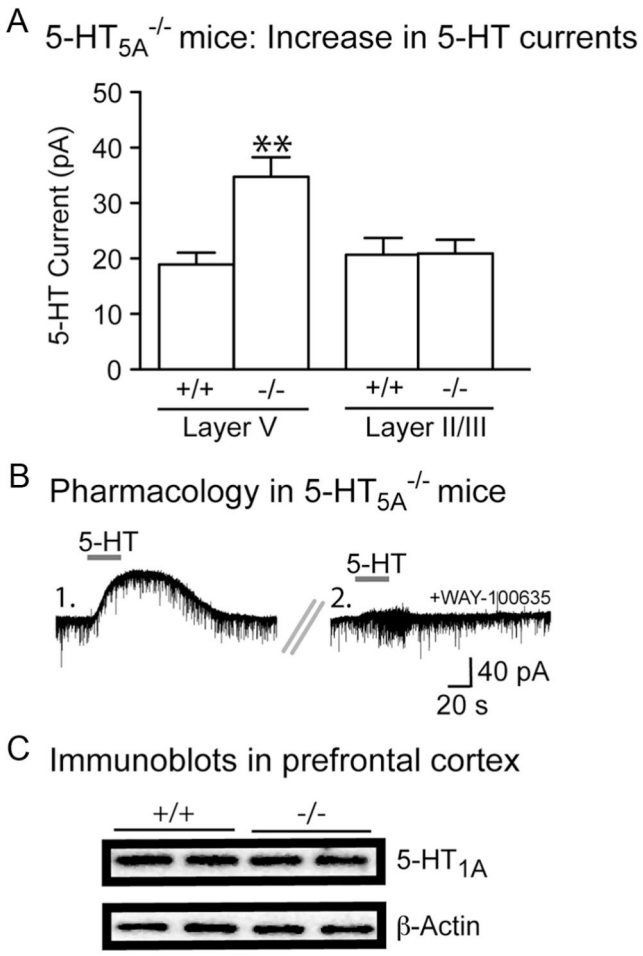

Figure 4.

5-$HT_{5A}^{-/-}$ mice display a selective upregulation of layer V 5-HT_{1A} currents, but not of prefrontal 5-HT_{1A} protein content. ***A***, In the absence of antagonists, the amplitude of the5-HT outward current is significantly larger in layer V neurons from 5-$HT_{5A}^{-/-}$ mice compared with layer V neurons from 5-$HT_{5A}^{+/+}$ mice or layer II/III neurons from either 5-$HT_{5A}^{+/+}$ or 5-$HT_{5A}^{-/-}$ mice (two-way ANOVA, significant interaction; $^{**}p = 0.01$). ***B***, Voltage clamp traces illustrate that the larger 5-HT outward current observed in 5-$HT_{5A}^{-/-}$ mice (1.) is completely suppressed by the selective 5-HT_{1A} receptor antagonist, WAY-100635 (2.). ***C***, Representative immunolabeling from two 5-$HT_{5A}^{+/+}$ and two 5-$HT_{5A}^{-/-}$ mice illustrating that prefrontal 5-HT_{1A} protein content is not significantly affected by genotype.