Brief Communications

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

Nathalie M. Goodfellow,[1] Craig D. C. Bailey,[1] and Evelyn K. Lambe[1,2]

[1]Department of Physiology and [2]Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada

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).

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.

Received Sept. 22, 2011; revised Feb. 27, 2012; accepted March 7, 2012.

Author contributions: N.M.G., C.D.C.B., and E.K.L. designed research; N.M.G. performed research; N.M.G., C.D.C.B., and E.K.L. analyzed data; N.M.G., C.D.C.B., and E.K.L. wrote the paper.

This work was supported by an NSERC Discovery Grant (E.K.L.), the Scottish Rite Charitable Foundation (E.K.L.), and the Canada Research Chairs program (E.K.L.). N.M.G. was supported by a Margaret Santalo fellowship and CIHR Banting and Best Doctoral award. We thank Dr. Rene Hen of Columbia University for the generous gift of the transgenic mice and the knock-out primer sequences, and Dr. Jeff Muller for facilitating the transfer of these mice. We thank Dr. Beverley Orser of the University of Toronto for the use of the imaging facility and Drs. Milton Charlton, Sabine Cordes, and Paul Fletcher for constructive suggestions on the manuscript.

Correspondence should be addressed to Dr. Evelyn K. Lambe, Department of Physiology, University of Toronto, 1 King's College Circle, Toronto, ON, M5S 1A8, Canada. E-mail: evelyn.lambe@utoronto.ca.

DOI:10.1523/JNEUROSCI.4849-11.2012

 0270-6474/12/325804-06$15.00/0

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′-TTTCTAGCTGCGGCCACA TTCACT-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′-ATTCGGCTATGACTGG GCACAACA-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 buf-

fer (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 from 5-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 pres-

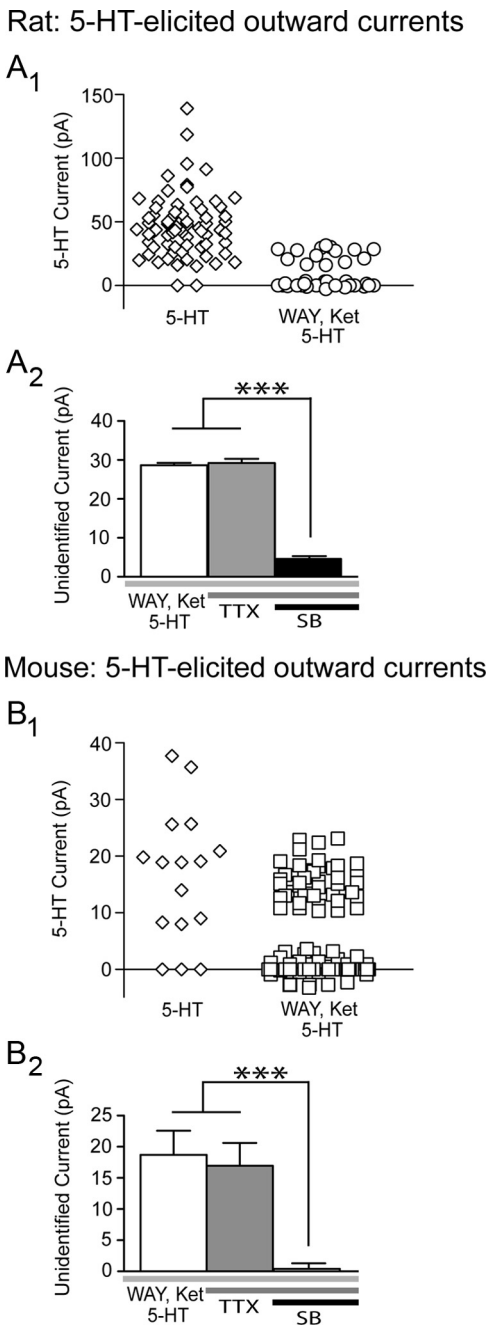

Figure 1. An unidentified 5-HT current in prefrontal cortex of rat and mouse: evidence of functional 5-HT$_{5A}$ receptors. In the rat (***A*$_1$**) and mouse (***B*$_1$**) prefrontal cortex, bath application of 5-HT (10 μM; 30 s) elicits an unidentified outward current in the presence of the 5-HT$_{1A}$ and 5-HT$_2$ antagonists, WAY-100635 (WAY) and ketanserin (Ket). The bar graphs summarize within-cell paired experiments from rats (***A*$_2$**) and mice (***B*$_2$**), 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, ***$p < 0.0001$).

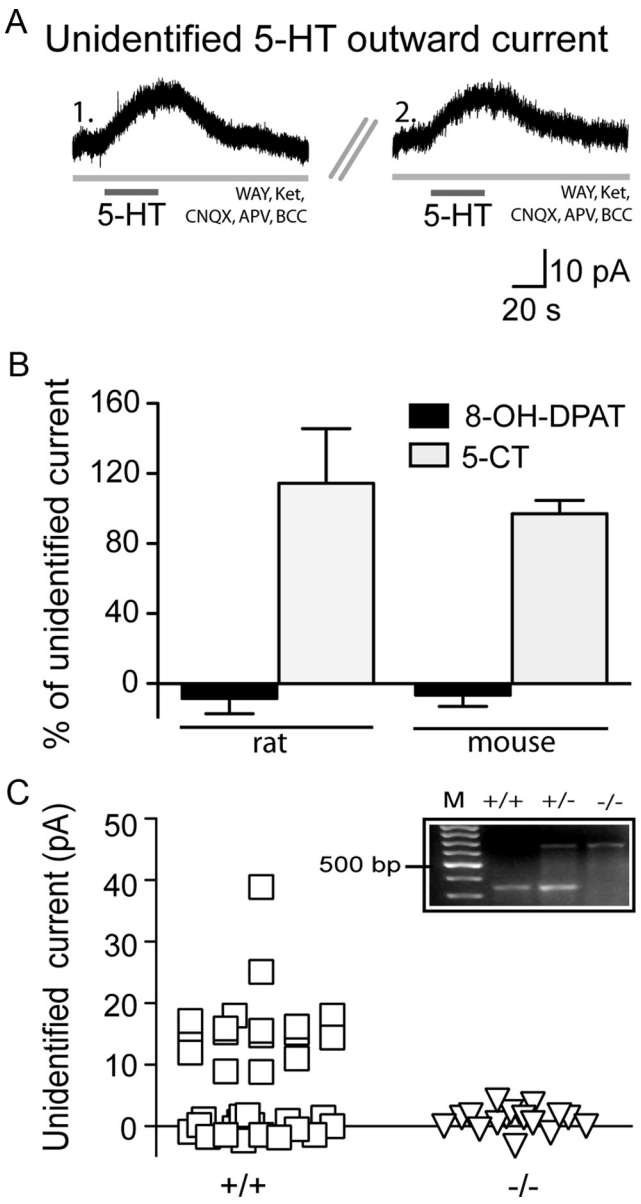

Figure 2. Pharmacological and transgenic confirmation that the 5-HT$_{5A}$ receptor mediates the unidentified 5-HT current. ***A***, Voltage-clamp traces showing the unidentified 5-HT current in the presence of WAY-100635 (WAY) and ketanserin (Ket) (1.) can be re-elicited upon repeat application of 5-HT 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.

ence 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 neu-

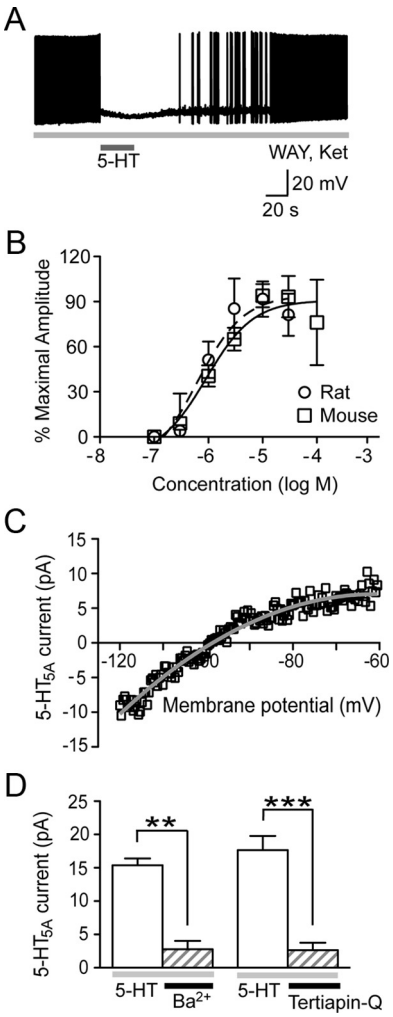

Figure 3. Characterization of the 5-HT$_{5A}$ current in the normal adult rodent cortex. ***A***, Current-clamp trace illustrates that the 5-HT$_{5A}$ current can inhibit neuronal excitability resulting from a constant depolarizing current. ***B***, The concentration–response curves demonstrate that 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.

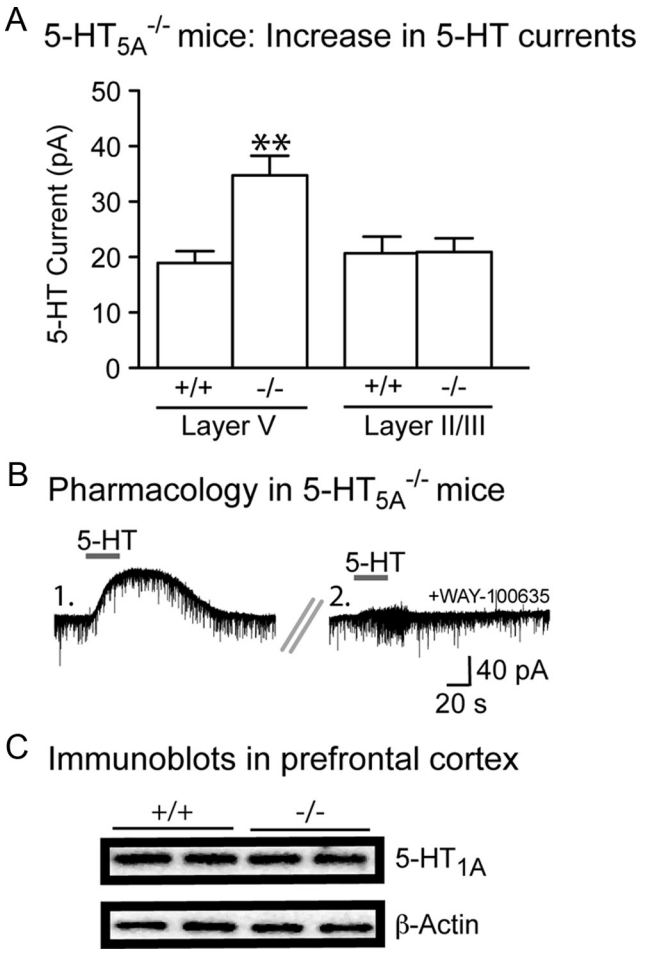

Figure 4. 5-HT$_{5A}^{-/-}$ mice display a selective upregulation of layer V 5-HT$_{1A}$ currents, but not of prefrontal 5-HT$_{1A}$ protein content. ***A***, In the absence of antagonists, the amplitude of the 5-HT outward current is significantly larger in layer V neurons from 5-HT$_{5A}^{-/-}$ mice 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.

ronal 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*B*$_1$ 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 supracompensatory 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 upregulation 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.

References

Amargós-Bosch M, Bortolozzi A, Puig MV, Serrats J, Adell A, Celada P, Toth M, Mengod G, Artigas F (2004) Co-expression and in vivo interaction of serotonin1a and serotonin2a receptors in pyramidal neurons of prefrontal cortex. Cereb Cortex 14:281–299.

Béïque JC, Campbell B, Perring P, Hamblin MW, Walker P, Mladenovic L, Andrade R (2004) Serotonergic regulation of membrane potential in developing rat prefrontal cortex: coordinated expression of 5-hydroxytryptamine (5-HT)1A, 5-HT2A, and 5-HT7 receptors. J Neurosci 24:4807–4817.

Belgard TG, Marques AC, Oliver PL, Abaan HO, Sirey TM, Hoerder-Suabedissen A, García-Moreno F, Molnár Z, Margulies EH, Ponting CP (2011) A transcriptomic atlas of mouse neocortical layers. Neuron 71:605–616.

Blier P, Ward NM (2003) Is there a role for 5-HT1A agonists in the treatment of depression? Biological Psychiatry 53:193–203.

Bruinvels AT, Landwehrmeyer B, Gustafson EL, Durkin MM, Mengod G, Branchek TA, Hoyer D, Palacios MJ (1994) Localization of 5-HT 1 B, 5-HT 1 Dα, 5-HT 1 E and 5-HT 1 F receptor messenger RNA in rodent and primate brain. Neuropharmacology 33:367–386.

Corbett DF, Heightman TD, Moss SF, Bromidge SM, Coggon SA, Longley MJ, Roa AM, Williams JA, Thomas DR (2005) Discovery of a potent and selective 5-ht5A receptor antagonist by high-throughput chemistry. Bioorg Med Chem Lett 15:4014–4018.

Gingrich JA, Hen R (2000) The broken mouse: the role of development, plasticity and environment in the interpretation of phenotypic changes in knockout mice. Curr Opin Neurobiol 10:146–152.

Goodfellow NM, Benekareddy M, Vaidya VA, Lambe EK (2009) Layer II/III of the prefrontal cortex: inhibition by the serotonin 5-HT1A receptor in development and stress. J Neurosci 29:10094–10103.

Grailhe R, Waeber C, Dulawa SC, Hornung JP, Zhuang X, Brunner D, Geyer MA, Hen R (1999) Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. Neuron 22:581–591.

Grailhe R, Grabtree GW, Hen R (2001) Human 5-HT(5) receptors: the 5-HT(5A) receptor is functional but the 5-HT(5B) receptor was lost during mammalian evolution. Eur J Pharmacol 418:157–167.

Hannon J, Hoyer D (2008) Molecular biology of 5-HT receptors. Behav Brain Res 195:198–213.

Hattox AM, Nelson SB (2007) Layer V neurons in mouse cortex projecting to different targets have distinct physiological properties. J Neurophysiol 98:3330–3340.

Jacobsen KX, Czesak M, Deria M, Le François B, Albert PR (2011) Region-specific regulation of 5-HT1A receptor expression by Pet-1-dependent mechanisms in vivo. J Neurochem 116:1066–1076.

Kinsey AM, Wainwright A, Heavens R, Sirinathsinghji DJ, Oliver KR (2001) Distribution of 5-ht(5A), 5-ht(5B), 5-ht(6) and 5-HT(7) receptor mRNAs in the rat brain. Brain Res Mol Brain Res 88:194–198.

Matthes H, Boschert U, Amlaiky N, Grailhe R, Plassat JL, Muscatelli F, Mattei MG, Hen R (1993) Mouse 5-hydroxytryptamine5A and 5-hydroxytryptamine5B receptors define a new family of serotonin receptors: cloning, functional expression, and chromosomal localization. Mol Pharmacol 43:313–319.

Noda M, Yasuda S, Okada M, Higashida H, Shimada A, Iwata N, Ozaki N, Nishikawa K, Shirasawa S, Uchida M, Aoki S, Wada K (2003) Recombinant human serotonin 5A receptors stably expressed in C6 glioma cells couple to multiple signal transduction pathways. J Neurochem 84:222–232.

Okuhara DY, Beck SG (1998) Corticosteroids alter 5-hydroxytryptamine1A receptor-effector pathway in hippocampal subfield CA3 pyramidal cells. J Pharmacol Exp Ther 284:1227–1233.

Otsuka T, Kawaguchi Y (2008) Firing-pattern-dependent specificity of cortical excitatory feed-forward subnetworks. J Neurosci 28:11186–11195.

Pasqualetti M, Ori M, Marazziti D, Castagna M, Nardi I (1998) Distribution of 5-HT2c and 5-ht5a receptor mRNA in human brain. Ann N Y Acad Sci 861:245.

Puig, MV, Gulledge, AT (2011) Serotonin and prefrontal cortex function: neurons, networks, and circuits. Mol Neurobiol 44:449–464.

Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci U S A 95:14476–14481.

Raymond JR, Mukhin YV, Gettys TW, Garnovskaya MN (1999) The recombinant 5-HT1A receptor: G protein coupling and signalling pathways. Br J Pharmacol 127:1751–1764.

Sprouse J, Reynolds L, Braselton J, Schmidt A (2004) Serotonin-induced phase advances of SCN neuronal firing in vitro: a possible role for 5-HT5A receptors? Synapse 54:111–118.

Thomas DR, Soffin EM, Roberts C, Kew JN, de la Flor RM, Dawson LA, Fry VA, Coggon SA, Faedo S, Hayes PD, Corbett DF, Davies CH, Hagan JJ (2006) SB-699551-A (3-cyclopentyl-*N*-[2-(dimethylamino)ethyl]-*N*-[(4′-{[(2-phenylethyl)amino]methyl}-4-biphenylyl)methyl]propanamide dihydrochloride), a novel 5-ht5A receptor-selective antagonist, enhances 5-HT neuronal function: evidence for an autoreceptor role for the 5-ht5A receptor in guinea pig brain. Neuropharmacology 51:566–577.

Villalobos C, Beique JC, Gingrich JA, Andrade R (2005) Serotonergic regulation of calcium-activated potassium currents in rodent prefrontal cortex. Eur J Neurosci 22:1120–1126.

Zhong P, Yan Z (2011) Differential regulation of the excitability of prefrontal cortical fast-spiking interneurons and pyramidal neurons by serotonin and fluoxetine. PLoS One 6:e16970.