ACS Chemical Neuroscience

Research Article

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Pharmacological Evidence for 5-HT$_6$ Receptor Modulation of 5-HT Neuron Firing *in Vivo*

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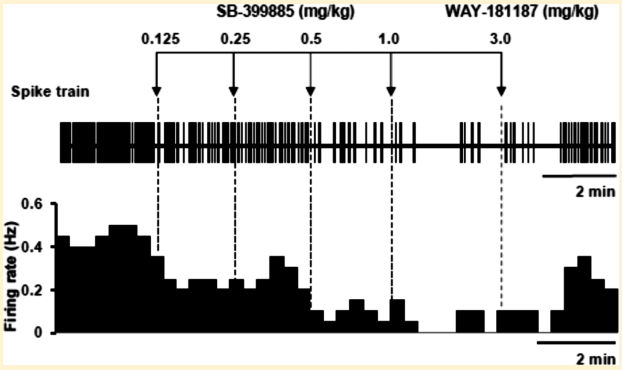
Supporting Information

ABSTRACT: 5-Hydroxytryptamine (5-HT) neurons in the midbrain dorsal raphe nucleus (DRN) are implicated in the drug treatment and pathophysiology of a wide variety of neuropsychiatric disorders. Accumulating evidence suggests that 5-HT$_6$ receptors may be located and functional in the DRN; therefore, 5-HT$_6$ receptor ligands may have potential as novel modulators of 5-HT neurotransmission. The current study investigated the effect of intravenous (i.v.) administration of the selective 5-HT$_6$ receptor agonist, WAY-181187, and antagonist, SB-399885, on the firing of 5-HT neurons in the DRN *in vivo*. Extracellular recordings were made in the DRN of anesthetized rats, and single 5-HT neurons were identified on the basis of electrophysiological properties combined with juxtacellular labeling and postmortem immunohistochemical analysis. WAY-181187 (1−4 mg/kg i.v.) caused a dose-dependent increase in 5-HT neuron firing rate. In comparison, SB-399885 (0.125−1 mg/kg i.v.) caused a dose-dependent decrease in 5-HT neuron firing rate, an effect reversed by WAY-181187 (3 mg/kg i.v.). These effects of WAY-181187 and SB-399885 were observed in two separate sets of experiments. In summary, the current data show the modulation of 5-HT neuronal firing by the 5-HT$_6$ ligands WAY-181187 and SB-399885 and are consistent with the presence of 5-HT$_6$ receptor-mediated positive feedback control of 5-HT neurons.



KEYWORDS: *5-Hydroxytryptamine, 5-HT, serotonin, 5-HT$_6$ receptor, dorsal raphe nucleus, electrophysiology*

The neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) is involved in many CNS functions ranging from emotion, cognition, learning, and memory mechanisms to regulation of the sleep−wake cycle and food intake.[1] Consequently, pharmacological modulation of 5-HT is associated with the symptomatic relief of numerous CNS disorders including major depression, anxiety, and schizophrenia,[1] although there is a large potential for improved 5-HT therapy of these and other illnesses.

The midbrain dorsal raphe nucleus (DRN) is rich in 5-HT neurons, which form the main site of origin of 5-HT projections to the forebrain. The DRN and its neural inputs are a rich source of drug targets to manipulate the 5-HT system,[2] and feedback mechanisms involving 5-HT receptors located both within and outside the DRN are important in this regard.[3] For instance, inhibitory mechanisms governing 5-HT neuron firing include feedback exerted by 5-HT$_{1A}$ autoreceptors within the DRN and a long-loop pathway mediated by 5-HT$_{1A}$ heteroceptors located postsynaptically outside of the DRN.[4] In addition, 5-HT$_{2A}$ and 5-HT$_{2C}$ heteroceptors located putatively in the prefrontal cortex and lateral habenula, respectively, act in cooperation with DRN GABAergic neurons to inhibit the firing of 5-HT neurons.[5,6] Evidence also suggests that 5-HT neurons are positively regulated by 5-HT$_4$ receptors. Thus, the firing of 5-HT neurons is increased by administration of 5-HT$_4$ receptor agonists, whereas antagonists or genetic knockout of the 5-HT$_4$ receptor has the opposite effect; a mechanism thought to involve the 5-HT$_4$ receptor modulation of an input to DRN 5-HT neurons from the prefrontal cortex.[7,8]

The 5-HT$_6$ receptor is one of the last 5-HT receptors to be discovered and does not yet have a well-defined functional role in the brain. However, emerging data suggest that this receptor may also modulate the activity of DRN 5-HT neurons, although the findings are not yet in full agreement. Recent receptor autoradiography studies reported moderate levels of 5-HT$_6$ receptor binding sites in the midbrain raphe region of the rat and marmoset, and these data are consistent with a previous quantitative RT-PCR study that found 5-HT$_6$ receptor mRNA in this area, although not located in 5-HT neurons.[9,10] However, earlier *in situ* hybridization and immunohistochemistry studies did not find detectable levels of 5-HT$_6$ receptor mRNA or protein in the midbrain raphe nuclei.[11,12] Never-

Special Issue: Serotonin Research

Received: February 12, 2015
Revised: April 1, 2015
Published: April 2, 2015

DOI: 10.1021/acschemneuro.5b00061
ACS Chem. Neurosci. 2015, 6, 1241−1247

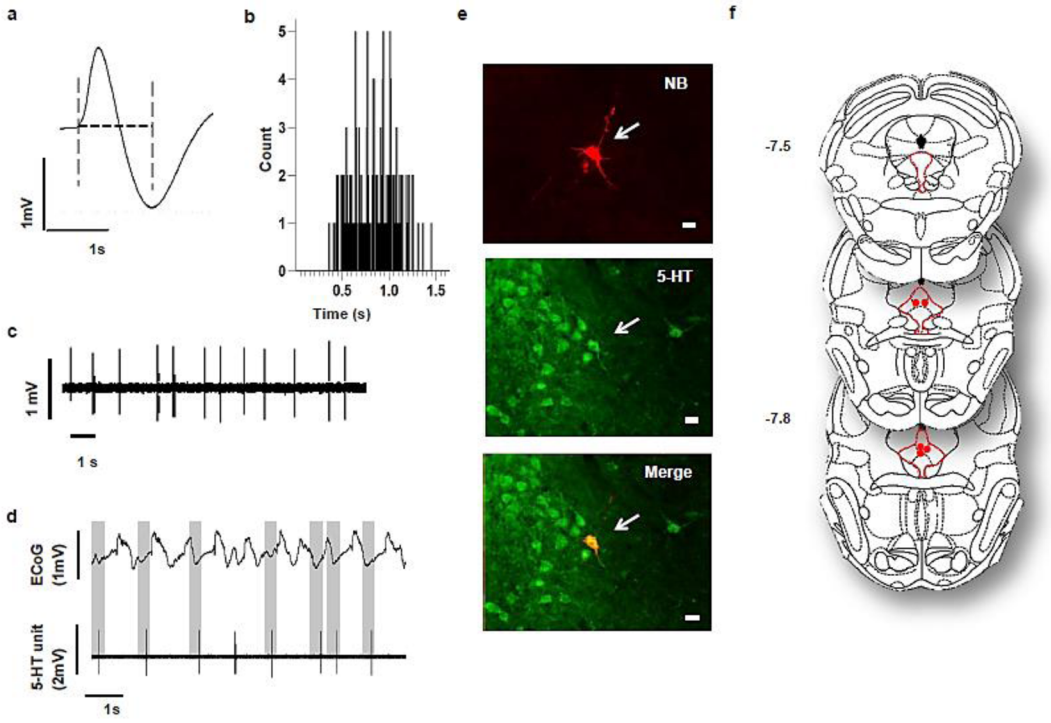


Figure 1. Electrophysiological and immunohistochemical properties of 5-HT neurons in the DRN of the urethane-anesthetized rat. Representative traces demonstrating (a) broad waveform, (b) regular interspike interval, and (c) regular and slow firing rates of a putative DRN 5-HT neuron. (d) Time-locked cortical ECoG (upper trace) and DRN single-unit recording (lower trace) showing coincidence of spike firing with the inactive phase of the ECoG. (e) Example of a juxtacellular-labeled, 5-HT-immunopositive DRN neuron: top panel, neurobiotin (NB); middle panel, 5-HT immunoreactivity; bottom panel, merged image (scale bar = 10 μm). (f) Coronal sections through the DRN showing location of juxtacellular-labeled 5-HT-immunopositive neurons (red dots); DRN outlined in red (coordinates according to Paxinos et al.[39]).

theless, all 5-HT_6 receptor localization studies agree that the receptor is abundant in forebrain regions such as the frontal cortex, which are well-known to exert a top-down influence on DRN 5-HT neurons.[13,3]

Functional evidence of an interaction between 5-HT_6 receptors and the DRN comes from a recent finding that systemic administration of the 5-HT_6 receptor agonist WAY-208466 increased wakefulness and that this could be mimicked by local injection of the drug into the DRN.[14] In addition to this, a report that WAY-181187 decreased extracellular levels of 5-HT in rat cortex, as measured by microdialysis, is consistent with an interaction between 5-HT_6 receptors and 5-HT neurons.[15] However, other microdialysis studies found no effect of various 5-HT_6 receptor ligands, including the selective antagonist SB-399885, on brain extracellular 5-HT.[16−18]

Finally, indirect evidence from studies on the effect of 5-HT_6 receptor ligands on 5-HT-dependent behaviors also suggests a putative interaction between 5-HT_6 receptor and 5-HT neurons. Thus, several studies report both antidepressant and anxiolytic effects of 5-HT_6 receptor agonists including WAY-181187 in rodent models.[19−21] Paradoxically, 5-HT_6 receptor antagonists such as SB-399885 are reported to have similar effects to 5-HT_6 receptor agonists in these tests.[22]

Overall, there is evidence suggesting an action of 5-HT_6 receptors on 5-HT neuronal activity, but inconsistencies in the literature do not yet allow clear conclusions to be drawn. To our knowledge, the effect of 5-HT_6 ligands on the electrical activity of 5-HT neurons has not yet been tested directly. Here, we aimed to investigate the effect of the selective 5-HT_6 receptor agonist WAY-181187 and the selective 5-HT_6 receptor antagonist SB-399885 on the firing of DRN 5-HT neurons *in vivo*.

RESULTS AND DISCUSSION

Electrophysiological Recording of DRN 5-HT Neurons. The current study measured spontaneously active neurons in the DRN of rats anesthetized with either urethane (majority of cases) or chloral hydrate (as specified) using extracellular single-unit recordings. We targeted neurons with electrophysiological characteristics identified as 5-HT containing.[23−25]

Specifically, putative 5-HT neurons were recorded with the following properties: broad waveform width (1.11 ± 0.46 ms, n = 34), slow firing rate (1.02 ± 0.45 Hz), and regular firing pattern (coefficient of variance of the interspike interval 0.35 ± 0.13). Examples of typical DRN recordings are presented in Figure 1. As expected of single 5-HT neurons,[26] their slow and regular firing rate displayed a significant coherence with slow wave (0.5−1.5 Hz) cortical activity (subpopulation of n = 8 neurons, $p < 0.05$) and with firing being in synchrony with the inactive phase (Figure 1d). Of the DRN neurons recorded with the above electrophysiological properties, five were successfully juxtacellular-labeled, and all were found to be 5-HT-immunopositive (Figure 1e & f), thereby confirming the 5-HT identity of the DRN neurons recorded.

Effect of 5-HT_6 Receptor Agonist WAY-181187 on 5-HT Neuron Firing. Initial experiments tested the effect on 5-HT neuronal activity of the selective 5-HT_6 agonist WAY-181187[15] (see later for details of its pharmacology). This drug increased the firing rate of DRN 5-HT neurons when administered in accumulating doses (1, 2, 3, 4 mg/kg intravenous (i.v.)), and the effect was dose-dependent

DOI: 10.1021/acschemneuro.5b00061
ACS Chem. Neurosci. 2015, 6, 1241−1247

compared to both predrug baseline firing (one-way ANOVA, $F_{(1.5,7.4)} = 7.98$, $p < 0.05$, $n = 6$) and vehicle-injected controls (two-way ANOVA, $F_{(1,12)} = 52.73$, $p < 0.001$), as illustrated in Figure 2. Firing rate increased by 58% above baseline at the highest dose tested (4 mg/kg i.v.), and there was no significant correlation between baseline firing rate and the magnitude of response to WAY-181187 (Pearson correlation, $r = -0.19$, $p > 0.05$).

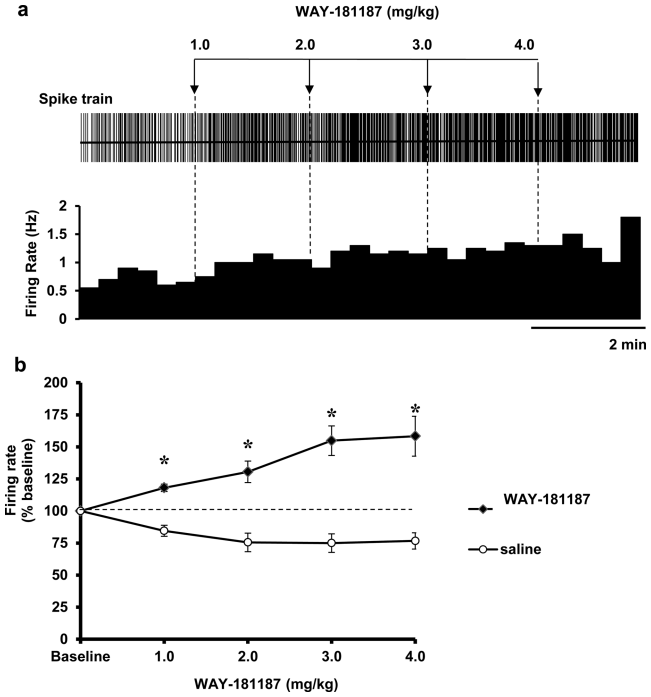


Figure 2. Effect of the 5-HT$_6$ receptor agonist WAY-181187 on 5-HT neuronal activity. (a) Spike train (upper trace) and rate-meter (lower trace) recordings of a single putative 5-HT neuron before and after i.v. administration of WAY-181187. (b) Group data showing the effect of WAY-181187 and vehicle. Mean ± SEM values; $n = 6$ rats; *$p < 0.05$ compared to baseline firing (one-way ANOVA). Predrug baseline firing rates were 1.02 ± 0.15 Hz (vehicle) and 0.76 ± 0.19 Hz (WAY-181187).

This experiment using urethane-anesthetised rats was repeated using a wider range of doses of WAY-181187 (0.1, 0.3, 1, 3 mg/kg i.v.; chloral hydrate anesthesia). This experiment also found that WAY-181187 caused a dose-related increase in firing rate (maximal increase of 70% above baseline, one-way ANOVA, $F_{(4,24)} = 6.69$, $p < 0.001$, $n = 7$; Supporting Information Figure S1). Therefore, the data as a whole support an excitatory effect of WAY-181187 on 5-HT neurons in the DRN that is reproducible and dose-dependent.

Previous studies found that doses of WAY-181187 similar to those used here elicited behavioral and neurochemical effects *in vivo*. For instance, 3 mg/kg subcutaneous (s.c.) WAY-181187 was active in both an antidepressant test as well as a model of conditioned place preference.[27,19] Furthermore, in microdialysis studies, 3 mg/kg s.c. WAY-181187 caused a 5-HT$_6$ receptor mediated increase in extracellular GABA in rat frontal cortex.[15]

Effect of 5-HT$_6$ Antagonist SB-399885 on 5-HT Neuronal Activity. We also examined the effect on 5-HT neuronal activity of the selective 5-HT$_6$ antagonist SB-399885. Preliminary experiments revealed that, in contrast to WAY-181187, SB-399885 decreased the firing of 5-HT neurons, but that the effect was greatest in neurons with the slower baseline firing rate (Pearson correlation, $r = 0.67$, $p < 0.05$). Subsequent experiments selected neurons with a slower firing rate (0.5−1.5 Hz) and showed that accumulating doses of SB-399885 (0.125, 0.25, 0.5, 1.0 mg/kg i.v.) produced a dose-dependent decrease in firing of 5-HT neurons compared to baseline levels (one-way ANOVA, $F_{(4,28)} = 10.36$, $p < 0.001$, with posthoc analysis showing significant effects at 0.5 and 1.0 mg/kg), with the greatest decrease being 56% below baseline levels at the highest dose (Figure 3). A two-way ANOVA comparing the effect of SB-399885 was borderline significance ($p = 0.06$, within contrast, time x treatment interaction $p = 0.1$) when compared to vehicle-injected controls, likely due to a gradual downward decrease in firing over time after vehicle (0.18% saline/4% glucose) injection. However, a significant difference in the firing rate was revealed when comparing SB-399885 (1 mg/kg) with vehicle controls (Student's unpaired t test $p < 0.05$; Figure 3b). In the same experiments, WAY-181187 (3 mg/kg i.v.) reversed the inhibitory effect of SB-399885, and this was also statistically significant (Student's paired t test $p < 0.05$; Figure 3).

The above experiments with SB-399885 were repeated (0.125, 0.25, 0.5, 1.0 mg/kg i.v.: chloral hydrate anesthesia), and the drug also reduced 5-HT neuron firing rate (with the greatest decrease being 65% below baseline levels; one-way ANOVA, $F_{(4,20)} = 20.26$, $p < 0.001$; Supporting Information Figure S1); furthermore, the effect was reversed by 3 mg/kg WAY-181187 ($p < 0.05$; Supporting Information Figure S1). In comparison to SB-399885, administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (10 μg/kg i.v.) completely abolished 5-HT cell firing ($n = 5$, data not shown), an expected response of 5-HT neurons to pharmacological activation of 5-HT$_{1A}$ autoreceptors.

Overall, these data show that, in contrast to the excitatory effect of the 5-HT$_6$ receptor agonist WAY-181187, SB-399885 decreased the firing of 5-HT neurons in the DRN and that this effect was reversed by WAY-181187.

Role of 5-HT$_6$ Receptors in the Effects of WAY-181187 and SB-399885. Of the currently available 5-HT$_6$ agonists (including WAY-208466 and ST-1936), WAY-181187 demonstrates one of the highest 5-HT$_6$ receptor affinity (K_i 2.2 nM) and selectivity (60-fold) over other 5-HT and monoamine receptors.[15] In addition to this, WAY-181187 stimulated the production of cAMP to a magnitude comparable with that of 5-HT (E_{max} 93%), making the drug a full 5-HT$_6$ receptor agonist.[15]

In support of the idea that 5-HT$_6$ receptors mediate the inhibitory effect of SB-399885, the drug demonstrates high affinity (K_i 9 nM) and 200-fold selectivity for the 5-HT$_6$ receptor over a range of other 5-HT and monoamine binding sites.[17] *Ex vivo* binding assays showed that 3 and 10 mg/kg intraperitoneal (i.p.) SB-399885 produced, respectively, 62 and 96% 5-HT$_6$ receptor occupancy in rat brain.[28] Although the highest dose of SB-399885 tested here was 1 mg/kg, the drug was administered intravenously and likely reached peak concentrations rapidly and with a much higher degree of receptor occupancy than the same dose of the drug administered intraperitoneally. Moreover, 1−3 mg/kg i.p. SB-399885 produced robust anxiolytic effects in various rat models.[29]

In addition to the high 5-HT$_6$ receptor affinity and selectivity of both WAY-181187 and SB-399885, the observation that WAY-181187 reversed the inhibitory effect of SB-399885 is consistent with the involvement of 5-HT$_6$ receptors in the

DOI: 10.1021/acschemneuro.5b00061
ACS Chem. Neurosci. 2015, 6, 1241−1247

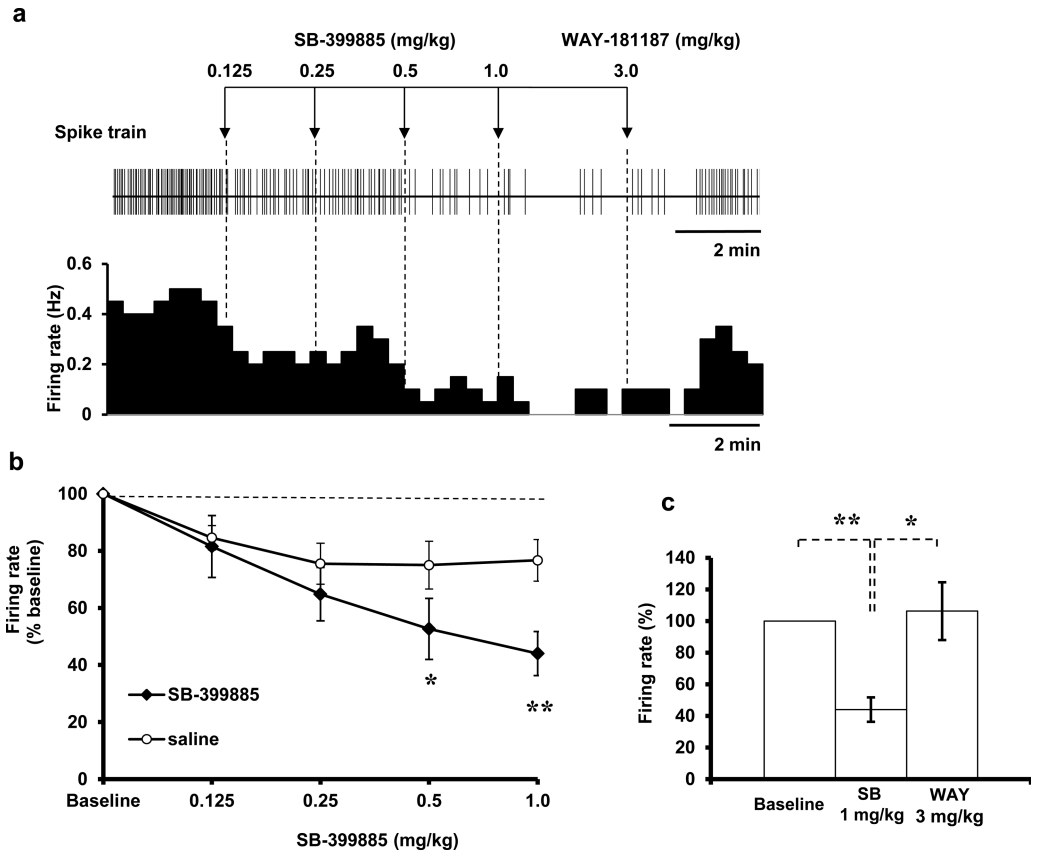


Figure 3. Effect of the 5-HT$_6$ receptor antagonist SB-399885 on 5-HT neuronal activity. (a) Spike train (upper trace) and rate-meter (lower trace) recordings of a single putative 5-HT neuron before and after i.v. administration of SB-399885. (b) Group data showing the effect of SB-399885 and vehicle. (c) Reversal of the inhibitory effect of SB-399885 by WAY-181187. Mean ± SEM values; n = 8 rats; *p < 0.05, **p < 0.01 compared to baseline firing (one-way ANOVA followed by paired Student's t test). Note the nonlinear x-axis, which is used for illustration purposes. Predrug baseline firing rates were 1.02 ± 0.15 Hz (vehicle) and 1.22 ± 0.29 Hz (SB-399885).

actions of these ligands. An interaction between SB-399885 and WAY-181187 has been observed in previous studies. In particular, in rats, SB-399885 reduced the cognition-enhancing effect of WAY-181187 as well as the associated activation of the prefrontal cortex.[30] Furthermore, SB-399885 attenuated WAY-181187-evoked changes in LTP in hippocampal slices.[31] In addition to these studies, SB-399885 has been shown to antagonize the actions of other 5-HT$_6$ receptor agonists. For instance, in rats, SB-399885 reversed the increase in wakefulness induced by the 5-HT$_6$ receptor agonist WAY-208466[14] and also prevented an increase in extracellular catecholamines evoked by the 5-HT$_6$ receptor agonist ST-1936.[18] The availability of a knockout model would help to resolve this question of the role of 5-HT$_6$ receptors. However, this would require the availability of a 5-HT$_6$ receptor knockout rat because the pharmacological profile of the mouse 5-HT$_6$ receptor is significantly different from that of the rat and human.[32]

In addition to pharmacological considerations, other indirect findings support a role for the 5-HT$_6$ receptor in the effects of WAY-181187 and SB-399885 on 5-HT neuron firing. In particular, this idea is consistent with reports of 5-HT$_6$ receptor localization in the midbrain raphe nuclei.[9,10] Also, local injection of 5-HT$_6$ receptor agonist WAY-208466 into the DRN elicits changes in sleep and wakefulness in rats.[14] Although a direct action in the DRN could be the neural site mediating the effects of the 5-HT$_6$ receptor ligands on 5-HT neuron firing rate, 5-HT$_6$ receptors are abundant in brain regions such as the prefrontal cortex that are important sources of neural inputs to the DRN.[3] Future studies aimed to test the effect of local injection of 5-HT$_6$ receptor ligands on the firing of DRN 5-HT neurons will help to clarify this issue.

A final point is that the current findings that WAY-181187 increased 5-HT cell firing, and that SB-399885 decreased 5-HT cell firing, are consistent with the presence of 5-HT$_6$ receptor-mediated positive feedback control of 5-HT neurons. Moreover, the effect of the 5-HT$_6$ receptor antagonist alone suggests that this feedback control is tonically active. This concept is remarkably similar to that proposed for 5-HT$_4$ receptors. Specifically, on the basis of evidence that 5-HT$_4$ receptor agonists and antagonists increase and decrease 5-HT cell firing, respectively, it is suggested that 5-HT$_4$ receptors exert a tonically active positive feedback control of 5-HT neurons.[33] Thus, 5-HT$_4$ and 5-HT$_6$ receptors might act in concert to provide a homeostatic positive feedback control of 5-HT neurons, whereas other 5-HT receptor subtypes, including 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, provide a balancing negative feedback control.

Possible Role of Changes in 5-HT Neuron Firing in Behavioral Effects of WAY-181187 and SB-399885. 5-HT$_6$ ligands including those tested here have behavioral effects that could plausibly link to changes in 5-HT cell firing. For instance, WAY-181187 and other 5-HT$_6$ receptor agonists are antidepressant as well as procognitive in animal models,[19,20,30] and, in theory, this could link to increased 5-HT neuron firing. Similarly, the reported anxiolytic effect of SB-399885 and other

DOI: 10.1021/acschemneuro.5b00061
ACS Chem. Neurosci. 2015, 6, 1241−1247

5-HT$_6$ receptor antagonists could conceivably be mediated by a decrease in 5-HT cell firing.[29,34]

However, this is likely to be an oversimplification because, paradoxically, both 5-HT$_6$ receptor agonists and antagonists are reported to be active in certain models of depression, cognition, and anxiety;[19,20,29,30,34−36] the explanation of this paradox is currently unclear, and reasons include currently unrecognized inverse agonism or biased agonism of the 5-HT$_6$ ligands as well as potential regional variations in 5-HT$_6$ tone and even constitutive activity. Another unknown is whether the changes in 5-HT neuron firing induced by WAY-181187 and SB-399885 observed here translate into a changes in 5-HT transmission at the nerve terminal. Thus, microdialysis studies report that WAY-181187 decreased extracellular 5-HT in rat cortex, whereas and SB-399885 had no effect.[15,17] However, regional differences in the effects of 5-HT$_6$ receptor ligands and incomplete sampling of the 5-HT neuron population herein could account for such differences. A final point, at least in the case of the antidepressant effects of SB-399885, is that depletion of 5-HT did not prevent this effect, suggesting a 5-HT-independent mechanism.[37]

CONCLUSIONS

In summary, the current study found that the selective 5-HT$_6$ receptor agonist WAY-181187 increased the firing rate of 5-HT neurons. In comparison, the selective antagonist SB-399885 decreased the firing rate of 5-HT neurons, an effect reversed by WAY-181187. The high selectivity of WAY-181187 and SB-399885 for the 5-HT$_6$ receptor implicates the involvement of this receptor in these electrophysiological effects. It is conceivable that changes in 5-HT neuron firing by 5-HT$_6$ ligands contribute to some of the behavioral effects of these agents, but this awaits confirmation. Overall, the present data constitute evidence for the presence of 5-HT$_6$ receptor-mediated positive feedback control of 5-HT neurons. Further pharmacological analysis of the influence of the 5-HT$_6$ receptor on 5-HT neuron is warranted to test this hypothesis.

METHODS

Animals. Male Sprague−Dawley rats (230−350 g, Harlan Laboratories, Bicester, UK) were housed at 20 ± 2 °C under 12 h light−dark cycles (lights on 0800 h) with water and food available *ad libitum*. Experiments complied with the Animal (Scientific Procedures) Act of 1986 and were approved by a local ethical review process at the University of Oxford.

Electrophysiological Recordings and Juxtacellular Labeling. Anesthesia was induced using isoflurane and maintained with either urethane (1.3 mg/kg i.p.) and supplemental doses of ketamine (30 mg/kg i.m. or i.p.) and xylazine (3 mg/kg i.m. or i.p.) or chloral hydrate (initial dose 460 mg/kg i.p. followed by supplementary doses of 60−120 mg/kg i.p. as required, without ketamine). Animals were placed in a stereotaxic frame, and body temperature was maintained at 37 °C with a homeothermic heating blanket. A tail vein was cannulated, a craniotomy was performed over the DRN, and skull screws were inserted over the frontal cortex and ipsilateral cerebellum (reference) for electrocorticogram (ECoG) recordings.

Glass microelectrodes (10−25 MΩ *in situ* resistance, ∼1 μm tip diameter) were filled with 2 M NaCl or, for labeling experiments, 0.5 M NaCl containing 1.5% neurobiotin (Vector Laboratories, Peterborough, UK) and lowered into the DRN using a microdrive (Inchworm, Burleigh). Extracellular AC-coupled signals were amplified by a head-stage (×1000; Cygnus, Pennsylvania, USA) and filtered (between 300 and 5000 Hz; NeuroLog System, Digitimer, Welwyn Garden City, UK). ECoG signals were also band-pass filtered (0.3−1500 Hz, −3 dB limits; NeuroLog System) and amplified (×2000, NeuroLog system). Both single-unit and ECoG recordings were then filtered at 50 Hz to remove mains noise (HumBug 50/60 Hz Noise Eliminator, Quest Scientific, Vancouver, Canada) and were monitored using Spike2 software (version 6.17; Cambridge Electronic Design, Cambridge, UK) via a Micro1401 analogue-digital converter Interface (Cambridge Electronic Design).

Once a neuron was detected, a stable baseline firing rate was established for 2 to 3 min, followed by drug injections (i.v.) at 2 min intervals. Typically, drugs were administered in accumulating doses to maximize data per animal and thereby reduce animal numbers. Attempts were then made to juxtacellular label the neuron with neurobiotin by passing positive current pulses (200 ms on/off, 1−10 nA) via the microelectrode, which typically caused entrainment that was maintained for 30 s or longer to ensure sufficient labeling.[24,25,38] Typically, juxtacellular labeling is difficult to perform in slow-firing or inactive neurons. Consequently, we avoided experiments where drug administration would lower the firing rate prior to juxtacellular labeling (for example, reversal of the 5-HT$_6$ receptor agonist by the antagonist was not attempted because this was predicted to decrease the firing rate of the 5-HT neurons). After labeling, animals were left for at least 1 h before transcardial perfusion with phosphate-buffered saline (PBS) solution followed by 4% paraformaldehyde (PFA). Brains were removed and later processed for 5-HT immunohistochemistry.

Immunohistochemistry. Perfused brains were stored overnight in 4% PFA at 4 °C and then transferred into 30% sucrose solution and stored at 4 °C. Brains were subsequently sectioned (20 μm) using a cryostat (−20 °C). Free-floating sections were then stained for 5-HT and neurobiotin using immunohistochemistry. Sections were washed in PBS, followed by 50 mM ammonium chloride to quench free aldehyde groups, and then PBS containing 0.25% Triton X-100 (PBS-X). Sections were then incubated, first for 1 h at room temperature under continuous agitation with PBS-X containing 6% donkey serum and then overnight at 4 °C with a rabbit anti-5-HT antibody (kindly provided by Prof. Harry Steinbusch, Maastricht) diluted in PBS-X with 2% donkey serum. Next, sections were washed in PBS-X and incubated for 1 to 2 h with Alexa Fluor 488-conjugated donkey anti-rabbit IgG (1:1000, Invitrogen, UK) and Alexa Fluor 594-conjugated streptavidin (1:1000, Invitrogen, UK) in PBS-X with 2% donkey serum. Sections then went through a series of washes (PBS-X, followed by PBS, and finally PB) and were mounted onto slides, coverslipped using Vectashield (Vector Laboratories, Peterborough, UK), and then examined using an epifluorescence microscope (DM-5000B, Leica Microsystems, Germany). Brightness and contrast of the images were adjusted using ImageJ software (version 1.47, ImageJ, National Institute of Health, Bethesda, MD, USA).

Data and Statistical Analysis. Interspike interval, coefficient of variation of the interspike interval, firing rate, and spike waveform width (time taken from a 5% increase from baseline to the first trough; Figure 1a) were calculated for all neurons. The firing rate following drug injections was normalized to the baseline firing rate, which was taken as 100%. Statistical analysis was carried out using IBM SPSS Statistics 21 software (IBM, Portsmouth, UK) and included one-way repeated measures analysis of variance (ANOVA) with the Student's paired *t* test (two-tailed) and two-way ANOVA with LSD posthoc test. Data are expressed as mean ± SEM values.

Drugs. All drugs (WAY-181187, 2-(1-{6-chloroimidazo[2,1-*b*]-[1,3]thiazole-5-sulfonyl}-1*H*-indol-3-yl)ethan-1-amine; SB-399885, *N*-(3,5-dichloro-2-methoxyphenyl)-4-methoxy-3-(1-piperazinyl)-benzenesulfonamide) were made from desiccated powders and dissolved in isotonic glucosaline (NaCl 0.18% w/v, glucose 4% w/v). Where necessary, solutions were sonicated until dissolved and adjusted to pH 7.4.

ASSOCIATED CONTENT

Supporting Information

Effect of the 5-HT6 receptor agonist WAY-181187 and antagonist SB-399885 on the firing of 5-HT neurons in the DRN of the chloral hydrate anesthetized rat. This material is available free of charge via the Internet at http://pubs.acs.org.

DOI: 10.1021/acschemneuro.5b00061
ACS Chem. Neurosci. 2015, 6, 1241−1247

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

5-HT, 5-hydroxytryptamine; DRN, dorsal raphe nucleus

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