Serotonergic modulation of neuronal activity in rat midbrain periaqueductal gray

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Jeong HJ, Lam K, Mitchell VA, Vaughan CW. Serotonergic modulation of neuronal activity in rat midbrain periaqueductal gray. J Neurophysiol 109: 2712–2719, 2013. First published March 20, 2013; doi:10.1152/jn.00790.2012.—Serotonin (5-HT) modulates pain and anxiety from within the midbrain periaqueductal gray (PAG). In the present study, the effects of 5-HT- and 5-HT1A subtype-selective agonists on rat PAG neurons were examined using whole cell patch-clamp recordings in brain slices. In voltage clamp, 5-HT produced outward and inward currents in distinct subpopulations of neurons that varied throughout different subregions of the PAG. The 5-HT1A agonist R(+)8-OH-DPAT (1 μM) produced outward currents in subpopulations of PAG neurons. By contrast, sumatriptan (1 μM) and other 5-HT1B/D/F subtype agonists had little or no postsynaptic activity. The 5-HT2A/C agonists DOI (3 μM) and TCB-2 (1 μM) produced inward currents in subpopulations of PAG neurons, and DOI enhanced evoked inhibitory postsynaptic currents via a presynaptic mechanism. In current clamp, both R(+)8-OH-DPAT and sumatriptan produced an excitatory increase in evoked mixed postsynaptic potentials (PSPs). In addition, R(+)8-OH-DPAT, but not sumatriptan, directly hyperpolarized PAG neurons. By contrast, the 5-HT1D agonist DOI depolarized subpopulations of neurons and produced an inhibitory decrease in evoked mixed PSPs. These findings indicate that 5-HT1A and 5-HT1B/D/F ligands have partly overlapping inhibitory effects on membrane excitability and synaptic transmission within the PAG, which are functionally opposed by 5-HT2A/C actions in specific PAG subregions.

serotonin; PAG; postsynaptic; presynaptic; IPSP

THE NEUROTRANSMITTER SEROTONIN (5-HT) modulates a number of important physiological functions, including fear, anxiety, depression, pain, and sleep. These are mediated by a family of G protein-coupled receptors (GPCRs), including Gs-coupled 5-HT1A/B/D/F receptors, Gq-coupled 5-HT2A/C receptors, and G12/13-coupled 5-HT3, 5-HT7, and 5-HT1F receptors as well as 5-HT3 ligand-gated ion channel (Barnes and Sharp 1999). The midbrain periaqueductal gray (PAG), like the adjacent serotonergic dorsal raphe nucleus (DRN), contains a dense plexus of serotonergic nerve terminals (Clements et al. 1985).

Many functions of the PAG overlap those of the serotonergic system. The PAG plays a pivotal role in integrating an animal’s analgesic, somatomotor, autonomic, and behavioral responses to threat, stress, and pain (Keay and Bandler 2001). These responses are organized by functionally and anatomically distinct rostrocaudally aligned columns within the PAG. Briefly, actively coping strategies are organized by the dorsolateral and lateral columns, whereas passive coping strategies are organized by the ventrolateral lateral (Keay and Bandler 2001).

METHODS

Slice preparation. Experiments were carried out on male and female Sprague-Dawley rats (15–24 days old) following the guidelines of the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and with the approval of the Royal North Shore Hospital Animal Care and Ethics Committee. Animals were deeply anaesthetized with isoflurane and decapitated, and coronal midbrain slices (300 μm) containing PAG were cut using a vibratome (VT1000S; Leica Microsystems, Nussloch, Germany) in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 126 NaCl, 2.5 KCl, 1.4 NaH2PO4, 1.2 MgCl2, 2.4 CaCl2, 11 glucose, and 25 NaHCO3, as described previously (Drew et al. 2008). The slices were maintained at 34°C in a submerged chamber containing ACSF equilibrated with 95% O2 and 5% CO2. Individual slices were then transferred to a chamber and superfused continuously (1.8 ml/min) with ACSF at 34°C.

Electrophysiology. PAG and DRN (medial subdivision) neurons were visualized using Dodt-tube contrast gradient optics on an upright microscope (BX50; Olympus, Tokyo, Japan). Whole cell recordings were made using an Axopatch 200B (Molecular Devices, Sunnyvale,
CA). In voltage-clamp experiments examining postsynaptic actions, the internal solution comprised (in mM): 95 K-glucurate, 30 KCl, 15 NaCl, 2 MgCl₂, 10 HEPES, 2 MgATP, and 0.25 NaGTP. In voltage-clamp experiments examining effects on GABAergic synaptic transmission, the internal solution comprised (in mM): 140 K-glucurate, 10 HEPES, 11 EGTA, 2.4 MgCl₂, 2 MgATP, and 0.25 NaGTP. The internal solutions had a pH of 7.3 and osmolality of 280–285 mosmol/l, and liquid junction potentials were corrected. Series resistance (<30 MΩ) was compensated by 80% and continuously monitored during experiments.

In some experiments, electrically evoked postsynaptic currents and potentials (PSPs) were elicited in neurons via unipolar glass electrodes (containing ACSF) placed 50–150 μm lateral to the recording electrode (stimuli: 5–50 V, 20–400 μs). Voltage-clamp experiments, neurons were held at −60 mV, and GABA_A-, mediated evoked inhibitory postsynaptic currents (IPSCs) were elicited in ACSF containing the non-NMDA glutamate receptor antagonist CNQX (5 μM) and the glycine receptor antagonist strychnine (5 μM). In current-clamp experiments, the holding current was adjusted so that the initial membrane potential was 5–15 mV below action potential threshold, and PSPs were elicited in standard ACSF (as in Chiou and Huang 1999).

Analysis. Voltage-clamp and current-clamp recordings of membrane current and voltage, respectively, were filtered (2- and 10-kHz low-pass filters) and sampled (5 and 20 kHz) for later analysis (AxoGraph X; AxoGraph Scientific Software, Sydney, Australia). For voltage-clamp examination of direct postsynaptic effects, agonist-induced currents were measured from the top of the current trace. This avoided inclusion of IPSCs/excitatory postsynaptic currents (EPSCs) that were all inward with the K-glucurate/KCl-based internal recording solution [Fig. 1A, insets i and ii; IPSC and EPSC, reversal potential (E_rev) = −25 and +10 mV]. For voltage-clamp examination of effects on GABAergic synaptic transmission, the amplitude of evoked IPSCs (E_rev = 0 mV with CsCl-based internal solution) was measured as the peak of the IPSC relative to a 5-ms baseline period immediately before the stimulus artifact. For current-clamp recordings, the integral of evoked mixed PSPs was measured (integrated area under the PSP waveform) over a 100-ms period relative to a 5-ms baseline period immediately before the stimulus artifact (AxoGraph X). These mixed PSPs comprised both inhibitory and excitatory components (IPSPs and EPSPs, E_rev = −90 and +10 mV), and thus the integral of the evoked PSP had negative and positive components for IPSPs and EPSPs, respectively. Neurons in which action potentials occurred during evoked PSPs were excluded from the analysis of evoked PSP integral. Neurons were considered to be responders if there was a change in holding current >5 pA (voltage-clamp), a change in membrane potential >3 mV (current-clamp), or a change in the amplitude/integral of evoked IPSCs/PSPs >10%, which reversed on agonist washout or addition of an antagonist. All numerical data are expressed as means ± SE. Statistical comparisons of mean drug effects were made using Student’s paired t-test. Comparisons of proportions were made using χ² or Fisher’s exact tests. Differences were considered significant if P < 0.05.

Drug solutions. Stock solutions of all drugs were prepared in distilled water, or dimethyl sulfoxide, and then diluted (1:3,000 to 1:10,000, final vehicle concentration 0.01–0.033% vol/vol) to working concentrations using ACSF and applied by superfusion. Supramaximal concentrations of agonists and antagonists were used (as in Jeong et al. 2008). 1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrazol[3,2-b]pyridin-5-one dihydrochloride (CP 93129), 2-[5-[3-(4-methylsulfonylamino)benzyl]-1,2,4-oxadiazo-5-yl]-1H-indole-3-yl]ethanamine (L-694,247), N-(3R)-2,3-dimethylamino)-2,3,4,9-tetrahydro-1H-carbazole-6-yl]-4-fluoro-benzamide dihydrochloride (LY344864), (S)-3,4-dihydro-1-[2-[4-[4-(methoxyphosphonyl)-1-piperazinyl][ethyl]]-N-methyl-1H-2-benzopyran-6-carboxamide (PNU 109291), (2R)-(+)-1,8-hydroxy-2-(di-N-propylamino)-tetralin hydrobromide [(R)+-8-OH-DPAT], (4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylenemethyl hydrobromide (TCB-2), and N-(3R)-3-(tert-buty1)-3-(4-(2-methoxyphosphonyl)-1-piperazinyl)-1-yl]-2-phenylpropionamide dihydrochloride (WAY 100135) were obtained from Tocris Cookson (Bristol, United Kingdom); 6-cyano-O-methoxyphenoxaline-2,3-dione (CNQX), di(I-2-amino-5-phosphonopentanoic acid (AP5), QX-314 bromide, and TTX were from Ascent Scientific (Bristol, United Kingdom); (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride, (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride [(±)-DOI], 5-HT, methionine enkephalin, 3,2-(dimethylaminol)ethyl]-N-methyl-1H-indole-5-methanesulphonamide succinate (sumatriptan), N-[2-[4-(methylsulfonyl)-1-piperazinyl][ethyl]]-N2-pyridinylacyclohexanecarb-oxamide maleate (WAY 106635), and all other reagents were from Sigma-Aldrich (Sydney, Australia).

RESULTS

Postsynaptic actions of 5-HT in PAG. When neurons were voltage-clamped at −60 mV, administration of a maximal concentration of 5-HT (10 μM) onto midbrain slices produced

![Figure 1](https://example.com/figure1.png)
a range of postsynaptic current responses (Fig. 1, A–C). 5-HT produced an outward current in 48% of PAG neurons that reversed following washout (Fig. 1A; mean current = 30 ± 3 pA, n = 40). 5-HT produced an inward current in 20% of PAG neurons that slowly reversed following washout (Fig. 1C; mean current = −16 ± 3 pA, n = 16). In the other 32% of PAG neurons, 5-HT produced no change in membrane current (Fig. 1B; mean current = 0 ± 1 pA, n = 28). Subsequent application of the GABA<sub>B</sub> agonist baclofen (10 μM) produced an outward current that was not significantly different among the three types of 5-HT-responding neurons (Fig. 1, A–C; P = 0.2; mean current = 37 ± 3 pA, n = 84).

We examined whether these responses differed throughout subregions of the PAG and compared this with the adjacent DRN (Calizo et al. 2011; Marinelli et al. 2004). The relative proportions of 5-HT-responding types differed among neurons in the ventrolateral, lateral, and dorsolateral columns and the DRN (Fig. 1D; P = 0.01, \( \chi^2 = 16.1 \), degrees of freedom (df) = 6). This difference was largely due to a lower proportion of neurons that responded to 5-HT with an inward current in the ventrolateral PAG compared with the lateral and dorsolateral PAG and the DRN (Fig. 1D; P = 0.036, \( \chi^2 = 8.55 \), df = 3).

The endogenous opioid met-enkephalin (10 μM) produced an outward current in similar proportions of the three types of 5-HT-responding neurons (Fig. 2; Baclofen: mean current = 67% (13/19) and 80% (n = 8/10) of neurons that responded to 5-HT with outward and inward currents, respectively, and in 67% (n = 12/18) of neurons that did not respond to 5-HT. These findings indicate that 5-HT produces inward and outward currents in different subpopulations of PAG neurons that vary throughout the different columns but are independent of their opioid responsiveness.

**Actions of 5-HT<sub>1</sub> subtype-selective agonists.** We next examined the effect of a range of 5-HT<sub>1</sub> subtype-selective agonists on membrane current in PAG neurons. The 5-HT<sub>1A</sub> agonist R(+)-8-OH-DPAT (1 μM) produced an outward current in 53% of PAG neurons tested (Fig. 2, A and F; n = 18/34). The 5-HT<sub>1B</sub> agonist CP 93129 (1 μM) did not produce a change in membrane current in most neurons (Fig. 2B); producing an outward current in only 18% of PAG neurons (Figs. 3A and 2F; n = 4/22). Whereas the 5-HT<sub>1D</sub> agonist L-694,247 (1 μM) produced an outward current in 30% of PAG neurons (Figs. 2F and 3B; n = 3/10), another 5-HT<sub>1D</sub> agonist PNU 109291 (1 μM) did not produce a change in membrane current in any PAG neurons (Fig. 2, C and F; n = 8). The 5-HT<sub>1F</sub> agonist LY344864 (1 μM) did not produce a change in membrane current in any PAG neurons (Fig. 2, B and F; n = 12). The

![Fig. 2](https://example.com/fig2.png)

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**Fig. 2.** Postsynaptic actions of 5-HT<sub>1</sub> and<sub>2</sub> subtype-selective agonists. A–E: current traces of 5 different neurons during superfusion of the 5-HT<sub>1A</sub> subtype agonist R(+)-8-OH-DPAT (8-OH-DPAT; 1 μM), CP 93129 (CP; 1 μM), LY344864 (LY; 1 μM), PNU 109291 (PNU; 300 nM), and sumatriptan (Sum; 3 μM) as well as the 5-HT<sub>1A</sub> antagonist WAY 100635 (WAY; 3 μM) and the 5-HT<sub>1B</sub> subtype agonist TCB (TCB; 1 μM). In all neurons, baclofen (10 μM) was superfused following washout of the 5-HT ligands. F: bar chart of the percentage of neurons that responded to these 5-HT<sub>1A</sub> agonists and L-694,247 (L69; 1 μM; filled bars) and mean current produced in responding neurons (open bars). Neurons were voltage-clamped at −60 mV.

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antimigraine drug sumatriptan (1 μM) did not produce a change in membrane current in any PAG neurons (Fig. 2, D and F; n = 8). A higher concentration of sumatriptan (10 μM) produced an outward current in 6% of neurons (Fig. 2E; n = 1/18). It can be noted that R(+)8-OH-DPAT, CP 93129, and sumatriptan (1 μM) also produced a reduction in spontaneous synaptic currents even in cases where they did not produce a change in holding current (Fig. 2, A, B, and D).

In responding neurons, the outward current produced by R(+)8-OH-DPAT was reversed by addition of either of the 5-HT1A antagonists WAY 100135 (1 μM) or WAY 100635 (3 μM; Figs. 2A and 3C; n = 3 and 5; P = 0.001). The current induced by CP 93129 (1 μM) was unaffected by addition of the 5-HT1B antagonist NAS181 (3 μM) but was reversed following subsequent addition of WAY 100135 (3 μM; Fig. 3, A and C; n = 3; P < 0.05, Friedman test). Similarly, the current induced by L-694,247 (1 μM) was unaffected by addition of the 5-HT1D antagonist BRL15572 (1 μM) but was reversed following subsequent addition of WAY 100135 (3 μM; Fig. 3B; n = 1). The effect of antagonists in the one sumatriptan-responening neuron was not examined. These findings indicate that 5-HT1 ligands produce outward currents in PAG neurons that are largely mediated by 5-HT1A receptors.

**Actions of 5-HT2 subtype-selective agonists.** We next examined the effect of 5-HT2 subtype-selective agonists on membrane current in PAG neurons. The 5-HT2A agonist DOI (3 μM) produced an inward current in 43% of PAG neurons tested (Fig. 2F; n = 3/7). The 5-HT2A agonist TCB-2 (1 μM) produced an inward current in 17% of PAG neurons tested (Fig. 2, E and F; n = 2/12). Of the neurons tested with DOI and TCB-2, 50% (n = 2/4), 38% (n = 3/8), and 0% (n = 0/7) were in the dorsolateral, lateral, and ventrolateral PAG.

We have previously demonstrated that 5-HT inhibits GABAergic synaptic transmission within PAG and that this is mimicked by 5-HT1A receptor agonists as well as sumatriptan and 5-HT1B/D receptor agonists (Jeong et al. 2008). Given the above finding that 5-HT2A/C agonists have postsynaptic effects on PAG neurons, we also examined whether DOI modulates GABAergic synaptic transmission. In these experiments, we focused on the lateral and dorsolateral columns where 5-HT2 postsynaptic currents were observed. In these neurons, paired evoked IPSCs were elicited by two stimuli of identical strength in close succession (evoked IPSC2:1; interstimulus interval = 70 ms) in the presence of CNQX (5 μM) and strychnine (3 μM). Superfusion of DOI (3 μM) produced an increase in the amplitude of evoked IPSCs in subpopulations of lateral (n = 3/5) and dorsolateral (n = 1/4) PAG neurons that was significant when averaged across all neurons tested (Fig. 4, A and B; P = 0.03; n = 9). In these neurons, there was a decrease in the ratio of evoked IPSC2:1 (92 ± 3%, n = 9; P = 0.02; Fig. 4, A and B). These findings indicate that 5-HT2 ligands produce inward currents and enhance GABAergic synaptic transmission in subpopulations of lateral and dorsolateral PAG neurons.

Sero~tonergic effects on net neuronal excitability. In conjunction with prior studies, the above experiments suggest that the 5-HT1/2 receptor subtypes have distinct actions at pre- and postsynaptic sites. We next examined how these actions combined to alter net neuronal excitability by examining the effect of the 5-HT1/2 subtype-selective agonists R(+)8-OH-DPAT, sumatriptan, and DOI on basal and evoked neuronal excitability in current-clamp mode. In these experiments, a K-gluconate-based internal solution was used to examine the effect on membrane potential and evoked mixed PSPs.

![Figure 3](image-url)
and were blocked by picrotoxin (100 μM). Action potentials (Fig. 5A) are represented by filled and unfilled circles, respectively. In B, neurons in which DOI did and did not produce an increase in evoked IPSC1 amplitude are represented by filled and unfilled circles, respectively. In B, *P < 0.05. Neurons were voltage-clamped at –60 mV.

The 5-HT1/2 agonists had actions in current-clamp that were consistent with those observed in voltage clamp. R(+)-8-OH-DPAT (1 μM) hyperpolarized 83% of neurons tested (n = 10/12), and this was significant when averaged across all neurons (Fig. 5A and D; P = 0.0004). Sumatriptan (1 μM) had no effect on membrane voltage in any neuron tested (Fig. 5A, B and D; P = 0.9; n = 7). By contrast, DOI (3–10 μM) depolarized 33% of neurons tested (Fig. 5C and D; n = 3/9; P = 0.03), and in one neuron, this resulted in the generation of action potentials (Fig. 5C).

In these neurons, electrical stimulation evoked mixed PSPs that usually comprised overlapping IPSPs and EPSPs (Fig. 5A–C, and Fig. 6A–C). These IPSPs and EPSPs had varying kinetics and were blocked by picrotoxin (100 μM) and CNQX (10 μM), respectively. In most neurons, application of R(+)-8-OH-DPAT and sumatriptan produced an excitatory increase in the integral of the evoked mixed PSP that was significant when averaged across all neurons (Fig. 5E; P = 0.001, 0.01; n = 12, 6). The increase in the integral of the evoked mixed PSP was greater for sumatriptan than for R(+)-8-OH-DPAT (Fig. 5E; P = 0.03). This was usually observed as a reduction in the evoked IPSP and the unmasking of an evoked EPSP (Fig. 5A and B). It was possible that the increase in evoked mixed PSPs produced by R(+)-8-OH-DPAT was due to change in PSP driving force arising from the hyperpolarization. In five of the above neurons, R(+)-8-OH-DPAT produced an increase in the mixed PSP integral of 167 ± 17 mV ms when hyperpolarized (P = 0.0005). When the neurons were repolarized to the pre-R(+)-8-OH-DPAT value, the increase mixed PSP integral was 103 ± 17 mV ms, which was less than that in the hyperpolarized state (P = 0.03) but still significant (P = 0.004). Thus the effect of R(+)-8-OH-DPAT on PSPs was not solely a consequence of membrane hyperpolarization. In contrast to the 5-HT1 ligands, DOI usually produced an inhibitory decrease in the integral of evoked mixed PSPs in most neurons, which was observed as an increase in evoked IPSPs (Fig. 5C and E; n = 6/9; P = 0.04).

Given that 5-HT1A, 5-HT1B/D, and 5-HT2A/C agonists have differential pre- and postsynaptic effects, we then examined the net action of 5-HT on neuronal excitability. Application of 5-HT (10 μM) had a variable effect in current-clamp mode, hyperpolarizing 50% of neurons and depolarizing 25% of neurons tested (Fig. 6A–C and D; n = 8, 4). 5-HT had no effect on the resting membrane voltage in the other neurons (Fig. 6D; n = 4). In these neurons, 5-HT also had a variable effect on evoked mixed PSPs. The 5-HT-induced hyperpolarization was usually associated with an increase in the integral of evoked mixed PSPs (Fig. 6A, B, and D; n = 6/7). The increase in the evoked mixed PSP was usually observed as a reduction in IPSPs and the unmasking of EPSPs (Fig. 6A). Despite the membrane hyperpolarization, this led to an increase in transient action potential firing during the evoked PSP in two neurons (Fig. 6B). By contrast, the 5-HT-induced depolarization was always associated with an inhibitory decrease in the integral of evoked mixed PSPs and was observed as an increase in IPSPs (Fig. 6, C and D; n = 3/3).

**DISCUSSION**

The present findings suggest that 5-HT1A, 5-HT1B/D, and 5-HT2A/C receptor activation modulates neuronal excitability...
within the PAG via partly overlapping pre- and postsynaptic mechanisms. 5-HT$_{1A}$ and 5-HT$_{2A/C}$ receptors regulated PAG activity at the postsynaptic level, whereas 5-HT$_{1B}$ and 5-HT$_{2A/C}$ receptors modulated presynaptic inputs onto PAG neurons. In addition, the 5-HT$_1$ and 5-HT$_2$ receptor subtypes exerted functionally opposite actions at both pre- and postsynaptic levels.

In the present study, it was found that 5-HT produced either an outward inhibitory current (46%) or an inward excitatory current (18%) or had no postsynaptic effect (36%) in PAG neurons under voltage-clamp conditions. These proportions differ from that previously observed in acutely dissociated PAG neurons where 5-HT produced an outward inhibitory current in 35% of neurons and no inward excitatory currents were observed (Jeong et al. 2001). This difference may be due to the use of slices vs. acutely isolated neurons. It was also found that the proportions of 5-HT-responding types varied throughout the PAG. A greater proportion of neurons responded to 5-HT with an inward current in the lateral and dorsolateral PAG (26%) compared with the ventrolateral PAG (4%). It can be noted that 33% of neurons in the DRN, which is adjacent to the ventrolateral PAG, responded to 5-HT with an inward current in the lateral and dorsolateral PAG (26%) compared with the ventrolateral PAG (4%). It can be noted that 33% of neurons in the DRN, which is adjacent to the ventrolateral PAG, responded to 5-HT with an inward current as observed previously (Beck et al. 2004; Craven et al. 2001; Kirby et al. 2003; Liu et al. 2000; Marinelli et al. 2004; Xu et al. 1998). These observations indicate that there is a high degree of organization of postsynaptic 5-HT actions within the PAG.

The 5-HT$_1$-induced outward and inward currents were likely to be mediated by 5-HT$_{1A}$ and 5-HT$_{2A/C}$ receptors, respectively. The 5-HT$_{1A}$-selective agonist R(+)8-OH-DPAT produced an outward current under voltage-clamp conditions that was reversed by addition of the 5-HT$_{1A}$ antagonists WAY 100135 and WAY 100635. This inhibitory action is similar to the activation of a postsynaptic inwardly rectifying K$^+$ conductance by the less active isomer 8-OH-DPAT observed previously in PAG neurons (Jeong et al. 2001). The 5-HT$_{1B}$ agonist CP 93129 and the 5-HT$_{1D}$ agonist L-694,247 produced an outward current in only a small proportion of PAG neurons, whereas another 5-HT$_{1D}$ agonist, PNU 109291, and the 5-HT$_{1F}$ agonist LY344864 had no effect in any PAG neurons. In addition, the antimigraine drug sumatriptan, which is an agonist at 5-HT$_{1B/D}$ and 5-HT$_{1F}$ receptors, had no effect at a 1 µM concentration and produced an outward current in only 1/18 neurons at a 10 µM concentration. The CP 93129- and L-694,247-induced currents were likely to be 5-HT$_{1A}$ receptor-mediated because they were unaffected by 5-HT$_{1B}$ and 5-HT$_{1D}$ antagonists and were reversed by 5-HT$_{1A}$ antagonists. Like prior in vitro electrophysiological studies, the 5-HT$_1$ subtype agonists were used at concentrations in the low micromolar range. Although these 5-HT$_1$ subtype-selective agonists have low nanomolar affinities for their respective receptors, they have variable selectivity over other 5-HT$_1$ receptor subtypes. For example, PNU 109291 has >1,000-fold selectivity for 5-HT$_{1D}$ vs. 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, but sumatriptan (5-HT$_{1B/D}$ vs. 5-HT$_{1A}$), CP 93129 (5-HT$_{1B}$ vs. 5-HT$_{1A}$ and 5-HT$_{1D}$), and L-694,247 (5-HT$_{1D}$ vs. 5-HT$_{1A}$ and 5-HT$_{1B}$) have only an 8- to 200-fold selectivity for their respective targets (Beer et al. 1993; Ennis et al. 1998; Macor et al. 1990). This difference in selectivity was consistent with that observed in our study and indicates that the actions of 5-HT$_{1B/D}$ agonists should be confirmed with antagonists because they can produce 5-HT$_{1A}$ receptor-mediated effects at low micromolar concentrations. The 5-HT$_{2A}$ and 5-HT$_{2A/C}$ agonists TCB-2 and DOI both produced an inward excitatory current in subpopulations of PAG neurons, which is similar to that observed by others in the adjacent DRN (Calizo et al. 2011; Craven et al. 2001; Liu et al. 2000; Marinelli et al. 2004). In the present study, it was also shown that 5-HT$_{2A/C}$ receptor activation enhanced GABA$_A$-mediated synaptic transmission in a subpopulation of PAG neurons. This was likely to be mediated by a presynaptic mechanism because the increase in evoked IPSCs was associated with a change in their paired-pulse ratio. This differs from and would functionally oppose the 5-HT$_{1A}$- and 5-HT$_{1B/D}$-induced presynaptic inhibition that we and others have observed previously within the PAG and DRN (Jeong et al. 2008; Kishimoto et al. 2001; Lemos et al. 2006). It can be noted that this 5-HT$_{2A/C}$-mediated presynaptic enhancement was observed in the lateral and dorsolateral PAG,

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**Fig. 6.** 5-HT has differential effects on net neuronal excitability. **A–D:** voltage traces of 3 different neurons before (Pre) and during application of 5-HT (10 μM) in current-clamp mode. Scatterplots are of ΔV_m (D) and ΔPSP (E) produced by these agents. In **A–C,** electrical stimulation was applied at the arrow, stimulus artifacts have been blanked, and action potentials have been clipped for clarity. In **D** and **E,** responders and nonresponders are represented by filled and unfilled circles, respectively; the bar indicates the mean value for all neurons tested.
the columns in which the 5-HT2A/C-induced postsynaptic depolarization was most prominent. The actions of 5-HT2 ligands in the PAG therefore differ from the adjacent DRN where enhanced GABAergic synaptic transmission is solely due to postsynaptic depolarization and action potential-induced increases in IPSC frequency (Liu et al. 2000).

In conjunction with prior studies, the present voltage-clamp experiments indicate that different 5-HT receptors subtypes have distinct pre- and postsynaptic actions. Under current-clamp conditions, these distinct mechanisms combined to alter net neuronal excitability. The 5-HT1A and 5-HT1B/D agonists R(+)-8-OH-DPAT and sumatriptan both produced an excitatory increase in evoked mixed PSPs in subpopulations of neurons that in some cases resulted in transient action potential firing during the evoked PSP. The 5-HT1A and 5-HT1B/D agonist-induced excitatory increase in evoked mixed PSPs was likely to be presynaptically mediated because it was observed in the absence of postsynaptic changes in membrane voltage. These evoked mixed PSPs were a combination of GABAergic IPSPs and glutamatergic EPSPs (see also Chiou and Huang 1999). Thus the excitatory increase in the evoked mixed PSPs was likely to be due a reduction in GABAergic evoked IPSPs, which is mediated by presynaptic 5-HT1A and 5-HT1B/D receptor inhibition of GABA release (Jeong et al. 2008; Kishimoto et al. 2001). It can be noted, however, that 5-HT1A and 5-HT1B/D activation also presynaptically inhibits glutamate release within PAG (Jeong et al. 2008). This suggests that both 5-HT1A and 5-HT1B/D activation produce greater presynaptic inhibition of IPSPs compared with EPSPs, as observed previously for opioids within PAG (Chiou and Huang 1999). There were, however, subtle differences between the effects of R(+)-8-OH-DPAT and sumatriptan in current-clamp mode. First, the excitatory increase in evoked PSPs produced by sumatriptan was greater than that for R(+)-8-OH-DPAT. This is consistent with their contrasting actions on anxiety within the PAG columns and 5-HT receptor subtypes involved remain to be determined (Carstens et al. 1987; Nichols et al. 1989), although 5-HT1B/D receptors in the ventrolateral PAG have been implicated in analgesia specific to pain of intracranial origin (Bartsch et al. 2004; Knight and Goadsby 2001). The present study found that the ventrolateral PAG differs from the other PAG columns in that there is reduced 5-HT2A/C activity within this column. Thus, although functionally opposing 5-HT1A/B/D and 5-HT2A/C actions could potentially modulate analgesia within the lateral PAG, 5-HT1A/B/D actions will predominate in the ventrolateral PAG. Overall, the present study indicates 5-HT will have wide ranging roles in the modulation of pain and anxiety within this brain region and that subtype-selective ligands might be used to target specific functions within this brain structure.

Like opioids and cannabinoids, serotonergic systems within the PAG modulate pain and anxiety. In combination with prior studies, we have found that 5-HT1A/B/D and 5-HT2A/C receptor systems exert opposing postsynaptic actions and presynaptic modulation of GABAergic synaptic transmission in subpopulations of PAG neurons. These actions are more complex than those of opioids that directly inhibit a subpopulation of PAG neurons and both opioids and cannabinoids that presynaptically inhibit GABAergic and glutamatergic synaptic transmission throughout the lateral/ventrolateral PAG (Chieng and Christie 1994; Chiou and Huang 1999; Vaughan and Christie 1997; Vaughan et al. 2000). The present study suggests that 5-HT1A and 5-HT2A/C receptors have functionally opposing pre- and postsynaptic actions within the lateral/dorsolateral PAG, which is consistent with their contrasting actions on anxiety within these PAG columns (de Paula Soares and Zangrossi 2009; Graeff et al. 1993; Nogueira and Graeff 1991; Zanoveli et al. 2003). The presynaptic modulation of neuronal excitability by 5-HT1B/D receptors within these columns adds another component to anxiolytic control within this brain structure. Endogenous analgesia within the PAG is mediated via distinct non-opioid and opioid systems within the lateral/dorsolateral and ventrolateral PAG, respectively (see Hohmann et al. 2005). Although microinjection of 5-HT into the PAG has an analgesic action, the PAG columns and 5-HT receptor subtypes involved remain to be determined (Carstens et al. 1987; Nichols et al. 1989), although 5-HT1B/D receptors in the ventrolateral PAG have been implicated in analgesia specific to pain of intracranial origin (Bartsch et al. 2004; Knight and Goadsby 2001). The present study found that the ventrolateral PAG differs from the other PAG columns in that there is reduced 5-HT2A/C activity within this column. Thus, although functionally opposing 5-HT1A/B/D and 5-HT2A/C actions could potentially modulate analgesia within the lateral PAG, 5-HT1A/B/D actions will predominate in the ventrolateral PAG. Overall, the present study indicates 5-HT will have wide ranging roles in the modulation of pain and anxiety within this brain region and that subtype-selective ligands might be used to target specific functions within this brain structure.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


