SEROTONERGIC AND NON-SEROTONERGIC DORSAL RAPHE NEURONS ARE PHARMACOLOGICALLY AND ELECTROPHYSIOLOGICALLY HETEROGENEOUS


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Abbreviated title: Responses of dorsal raphe neurons to 5-HT

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ABSTRACT

The dorsal raphe nucleus (DRN) projects serotonergic axons throughout the brain and is involved in a variety of physiological functions. However, it also includes a large population of cells that contain other neurotransmitters. To clarify the physiological and pharmacological differences between the serotonergic and non-serotonergic neurons of the DRN, their postsynaptic responses to 5-hydroxytryptamine (5-HT, serotonin), to selective activation of 5-HT1A or 5-HT2A/C receptors and their action potential characteristics were determined using in vitro patch clamp recordings. The slices containing these neurons were then immunostained for tryptophan hydroxylase (TPH), a marker of serotonergic neurons. It was found that subpopulations of both serotonergic and non-serotonergic neurons (1) responded to 5-HT with outward (i.e. inhibitory) and inward (i.e. excitatory) currents, (2) responded to both 5-HT1A and 5-HT2A/C receptor activation with outward and inward currents, respectively, and (3) displayed overlapping action potential characteristics. These findings suggest that serotonergic and non-serotonergic neurons in the DRN are both heterogeneous with respect to their individual pharmacological and electrophysiological characteristics. The findings also suggest that the activity of the different populations of DRN neurons will display heterogeneous changes when the serotonergic tone in the DRN is altered by neurological disorders, or by drug treatment.

Keywords: serotonin; tryptophan hydroxylase; postsynaptic; patch clamp; immunohistochemistry.
INTRODUCTION

The dorsal raphe nucleus (DRN) projects to a wide range of midbrain and forebrain structures (Jacobs and Azmita 1992; Halliday et al. 1995). It is involved in the regulation of mood, sensory and motor functions and contains the largest group of serotonin (5-hydroxytryptamine; 5-HT)-containing neurons in the central nervous system. However, the DRN also contains a significant population of non-serotonergic neurons, including GABAergic neurons (Nanopoulos et al. 1982; Belin et al. 1983; Charara and Parent 1998) and it is unclear to what extent the responses of these groups differ.

Directly identified serotonin-containing DRN neurons have been characterized electrophysiologically as having broad action potentials, low firing rates and being inhibited (hyperpolarized) by serotonin via 5-HT1A receptor activation (Aghajanian and Vandermaelen 1982; Xu et al. 1998). By exclusion, putative non-serotonergic neurons have been described as those DRN neurons that have characteristics different from those described for serotonergic neurons (Vandermaelen and Aghajanian 1983). Numerous studies have used these pharmacological and electrophysiological criteria to indirectly identify putative serotonergic DRN neurons (e.g. Aghajanian and Lakoski 1984; Sprouse and Aghajanian 1987; Hajos et al. 1996; Liu et al. 2000; Craven et al. 2001; Haj-Dahmane 2001).

Interestingly, there is evidence that the activity of putative serotonergic and non-serotonergic DRN neurons may be modulated by both 5-HT1A and 5-HT2A/C postsynaptic receptors (Liu et al. 2000; Craven et al. 2001). Furthermore, recent studies have demonstrated that directly identified serotonergic and non-serotonergic DRN neurons both respond to 5-HT1A receptor agonists (Kirby et al. 2003; Beck et al. 2004). However, neurochemically defined serotonergic and non-serotonergic DRN neurons have not been examined for their responses to 5-HT, and to 5-HT1A and 5-HT2A/C subtype selective agonists, or for their action potential characteristics in any single study. In the present study, we have used a combination of in vitro electrophysiological and anatomical techniques to pharmacologically and electrophysiologically characterize DRN neurons that were also identified immunocytochemically as serotonergic or non-serotonergic.
METHODS

15-22 day old Sprague-Dawley rats were anaesthetised with halothane, decapitated and four to five coronal midbrain slices containing the dorsal raphe nucleus were cut (250-300 µm thick). The slices were cut in ice-cold artificial cerebrospinal fluid (ACSF) of composition: (mM): NaCl 126, KCl 2.5, NaH2PO4 1.4, MgCl2 1.2, CaCl2 2.4, glucose 11, NaHCO3 25. Slices were maintained at 34°C in a submerged chamber containing ACSF equilibrated with a mixture of 5% CO2 and 95% O2. The brain slices were then transferred to a chamber and superfused continuously (1.8 ml.min⁻¹) with ACSF (34°C).

Dorsal raphe neurons were visualized in the midline region ventral to the aqueduct in the caudal midbrain using infra-red Nomarski optics on an upright microscope (Olympus BX51). Whole-cell patch clamp recordings of trans-membrane currents were performed using patch electrodes (2-5 MΩ) filled with an internal solution containing 115mM K-gluconate, 25mM KCl 25; 15mM NaCl, 1mM MgCl₂, 10mM HEPES, 11mM EGTA, 2mM MgATP, 0.25mM NaGTP, 0.01% biocytin, pH 7.3, osmolarity 280-285 mosmol.l⁻¹. Series resistance (< 20MΩ) was compensated by 80% and continuously monitored during experiments with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA). Postsynaptic currents (under voltage clamp, holding potential -60mV, with a liquid junction potential correction of -10mV) were filtered (100Hz LPF) and sampled (500Hz) for later analysis (Axograph 4, Axon Instruments). Action potentials recorded under current clamp were filtered (20kHz LPF) and sampled (50kHz) for analysis. The action potential duration was measured from the threshold of the rapid polarising phase to an equivalent voltage on the repolarising phase (see horizontal dashed lines in Figures 1Aii, 2Aii). The presence of an inflection/hump on the repolarising phase of the action potential was assessed by visual inspection (see * in Figure 1Aii) and confirmed by the presence of an inflection on the differentiated action potential trace. The presence of a fast-afterhyperpolarisation (fast-AHP) immediately following the action potential, which was distinct to a longer lasting slow-AHP was visually assessed (see * in Figure 2Aii, 2Bii). The amplitude of the fast and slow AHPs were measured from the threshold of the rapid polarising phase (see vertical and horizontal dashed lines in Figures 1Aii, 2Aii, 2Bii).
Biocytin, 5-CT (5-carboxamidoytryptamine maleate), DOI (R(-)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride) and 5-HT (5-hydroxytryptamine creatinine sulfate) were obtained from Sigma (Sydney, Australia). Ketanserin (3-[2-[4-(4-Fluorobenzoyl)-0-piperidinyl]ethyl]-2,4[1H,3H]-quinazolinedione tartrate) and pindolol (1-(1H-Indol-4-yloxy)-3-[(1-nethylethyl)amino]-2-propanol) were from Tocris Cookson (Bristol, UK). All stock solutions for in vitro experiments were made in distilled water except for pindolol, which was made in DMSO. These solutions were diluted at working concentrations in the extracellular solution immediately before use and applied by superfusion.

Patch clamp recordings were made from 1 – 2 neurons per brain slice and recordings from each neuron lasted no more than 20 minutes. After recording the slices which containing biocytin-filled cells were fixed for 30-60 min in a phosphate buffered paraformaldehyde/picric acid solution (75mM KH$_2$PO$_4$; 85mM Na$_2$HPO$_4$; 4% (w/v) paraformaldehyde; 14% (v/v) saturated aqueous picric acid; pH 6.9). The slices were then washed 6-8 times and stored in a phosphate buffered sucrose solution (30mM KH$_2$PO$_4$; 70mM Na$_2$HPO$_4$; 10% sucrose (w/v); 0.01% (w/v) sodium azide; 0.032% (w/v) bacitracin; pH 7.2). Brainstem slices and spinal cords were shipped by courier from Sydney to Minneapolis for the anatomical portion of the experiments. Biocytin-filled cells were visualized by incubation with Cy5-labeled streptavidin (Jackson ImmunoResearch, West Grove, PA, USA). Tryptophan hydroxylase immunoreactivity (TPH-ir) was visualized using a sheep anti-TPH antiserum (Chemicon, Temecula, CA, USA) followed by Cy2-conjugated donkey anti-sheep IgG (Jackson ImmunoResearch). Images of filled cells were collected using a Bio-Rad MRC 1000 or MRC 1024 confocal microscope. All numerical data are expressed as means ± S.E.M, and statistical comparisons were made using $\chi^2$ tests for differences among proportions, or unpaired t-tests for comparing means. Differences were considered significant at a p value of <0.05.
RESULTS

Serotonergic responses and action potential characteristics of DRN neurons

Whole cell patch recordings were made from 154 dorsal raphe neurons voltage clamped at –60 mV. Superfusion of 5-HT (30 µM) produced an outward current in 77 % of DRN neurons tested (Figure 1a,b, mean current = 26 ± 2 pA, n = 123). 5-HT produced an inward current in 20 % of DRN neurons (Figure 2a,b, mean current = -23 ± 3 pA, n = 31). Below, neurons that responded to 5-HT with an inward current are referred to as '5-HT inward' neurons, while those that responded with an outward current are referred to as '5-HT outward' neurons. 5-HT had no effect on membrane current in the other DRN neurons (3 %, n = 5), however, these neurons responded with an outward current (32 ± 8 pA) to subsequent application of the NOP agonist nociceptin (300 nM).

We next examined the responses of DRN neurons to 5-HT sub-type selective agonists and antagonists. The 5-HT induced outward current was abolished by co-application of the 5-HT1A/B antagonist pindolol (1 - 3 µM, n = 14), or the 5-HT1A/7 antagonist NAN-190 (100 nM, n = 5). Both the 5-HT1A/B/D/7 agonist 5-carboxytryptamine (5-CT, 100 nM, n = 80/89) and the 5-HT1A/7 agonist 8-hydroxy-DPAT (300 nM, n = 6/6) produced outward currents in most DRN neurons, which were abolished by co-application of either pindolol (1-3 µM, n = 46; Figures 1a, 2b), or NAN-190 (100 nM, n = 7). The 5-HT induced inward current was abolished by co-application of the 5-HT2A/C antagonist ketanserin (1 - 3 µM, n = 7). The 5-HT2A/C partial agonist DOI produced an inward current in 55% (n = 16/29) of neurons tested and this current was abolished by co-application of ketanserin (1-3 µM, n = 13; Figures 1a, 2). These observations are consistent with prior studies which have demonstrated that the outward and inward currents produced by 5-HT are mediated by 5-HT1A and 5-HT2A/C receptors within the DRN, respectively (Sprouse and Aghajanian 1987; Xu et al. 1998; Liu et al. 2000; Craven et al. 2001; Kirby et al. 2003; Beck et al. 2004).

In some of the above experiments the action potential characteristics of DRN neurons were studied in current-clamp mode (Figures 1ii, 2ii, n = 78). DRN neurons displayed a range of action potential characteristics. The mean action potential duration was 2.5 ± 0.1 ms (range = 1.1 – 5.3 ms). 50 % of
neurons displayed an inflection on the repolarising phase (n = 39). 64% of neurons displayed a fast after-hyperpolarisation (AHP) following the action potential which had a mean amplitude of 29 ± 1 mV (range = 12 – 47 mV, n = 50). All DRN neurons displayed a slow AHP which had a mean amplitude of 24 ± 1 ms (range 14 – 38 mV).

**TPH immunoreactivity and serotonergic responses of DRN neurons**

Following the above recordings, 81 biocytin-filled DRN neurons were recovered and examined for tryptophan hydroxylase (TPH) immunoreactivity, a marker of serotonergic neurons. Of the recovered biocytin-filled DRN neurons 72% were immunoreactive for TPH (n = 58/81). Both TPH-positive and TPH-negative DRN neurons responded to 5-HT with outward and inward currents (Figures 1, 2), although TPH-positive and TPH-negative neurons differed in their proportions of 5-HT outward and inward neurons ($\chi^2 = 9.0$, $p < 0.005$). Of the TPH-positive neurons, 88% (n = 50/57) were 5-HT outward neurons and 12% (n = 7/57) were 5-HT inward neurons (Figure 3a). Of the TPH-negative neurons, 53% (n = 10/19) were 5-HT outward neurons and 42% (n = 8/19) were 5-HT inward neurons (Figure 3a). The other TPH-negative neuron did not respond to 5-HT. Furthermore, the mean outward current produced by 5-HT was greater in TPH-positive neurons (28 ± 3 pA) than in TPH-negative neurons (19 ± 3 pA, $p < 0.05$), although the mean inward current produced by 5-HT was similar in TPH-positive neurons (-19 ± 6 pA) and TPH-negative neurons (-21 ± 5 pA, $p > 0.05$). However, there was considerable overlap in the 5-HT induced current in TPH-positive and negative neurons (Figure 3b).

We next examined the TPH-immunoreactivity of neurons based upon their responses to 5-CT and DOI. The proportion of 5-CT and DOI responders was not significantly different between TPH-positive and TPH-negative neurons ($\chi^2 = 0.3$ and 1.0 respectively, $p > 0.05$). 5-CT elicited a response in 96% (n = 26/27) of the TPH-positive neurons and 100% (n=8/8) of the TPH-negative neurons (Figure 4a). DOI elicited a response in 47% (n = 7/15) of the TPH-positive neurons and 75% (n = 3/4) of the TPH-negative neurons. Furthermore, there was considerable overlap in the 5-CT and DOI induced currents in TPH-positive and negative neurons (Figure 4b). The 5-CT induced outward
current was similar in TPH-positive (42 ± 6 pA) and TPH-negative (42 ± 20 pA, p > 0.05) neurons which responded to 5-CT. The DOI induced inward current was also similar in TPH-positive (-10 ± 3 pA) and TPH-negative (-7 ± 2 pA, p > 0.05) neurons which responded to DOI.

In some of these neurons we examined the effect of both 5-CT and DOI (Figure 4c, n = 17). 62% (n = 8) of the TPH-positive neurons and 25% (n = 1) of the TPH-negative neurons responded exclusively to 5-CT. 8% (n = 1) of the TPH-positive neurons and none of the TPH-negative neurons responded exclusively to DOI. 31% (n = 4) of the TPH-positive neurons and 75% (n = 3) of the TPH-negative neurons responded to both 5-CT and DOI. Overall, 41% (n = 7) of the TPH-positive and TPH-negative neurons examined responded to both 5-CT and DOI (and these neurons included those which responded to 5-HT with outward (n = 4) and inward (n = 3) currents).

**TPH immunoreactivity and electrophysiological characteristics of DRN neurons**

We also examined the TPH-immunoreactivity of neurons based upon their action potential characteristics. The mean action potential duration of TPH-positive neurons (3.0 ± 0.3 ms, n = 20) was greater than that of TPH-negative neurons (2.0 ± 0.2 ms, n = 14, p < 0.005), although there was considerable overlap (Figure 5). TPH-positive and negative neurons could not be distinguished by other action potential characteristics, as follows. A similar proportion of TPH-positive (70 %, n = 14/20) and TPH-negative (43 %, n = 6/14) neurons displayed an inflection on their repolarising phase ($\chi^2 = 2.5, p > 0.05$). A similar proportion of TPH-positive (50 %, n = 10/20) and TPH-negative (57 %, n = 8/14) neurons displayed a prominent fast AHP ($\chi^2 = 0.2, p > 0.05$), which was of similar amplitude (26 ± 2 and 29 ± 4 mV, respectively, p > 0.05). A slow AHP was observed in all TPH-positive (23 ± 1 mV) and TPH-negative (23 ± 2 mV) neurons which was of similar amplitude (p > 0.05). In addition to action potential characteristics, we also examined the TPH-immunoreactivity of neurons based upon their membrane capacitance, as an indirect measure of soma/dendritic field size. The mean membrane capacitance of TPH-positive neurons (39 ± 3 pF, n = 38) was not significantly different to that of TPH-negative neurons (33 ± 3 ms, n = 17, p > 0.05).
We next examined TPH immunoreactivity in DRN neurons which were characterized by both their action potential duration and by their response to 5-HT (n = 29). All of the 5-HT outward neurons with action potential durations > 2.5 ms were TPH-positive (n = 13/13). Conversely, 86% of the 5-HT inward neurons with action potential durations < 2.0 ms were TPH-negative (n = 6/7). Neurons with intermediate action potential durations (from 2.0 - 2.5 ms) were mostly 5-HT outward neurons (n = 5/6, the other did not respond to 5-HT) and were either TPH-positive (n = 3), or TPH-negative (n = 3). Neurons with other combinations of 5-HT responses and action potential duration were either TPH-positive (n = 2), or TPH-negative (n = 1).

**Distribution of cells**

The cells included in this study were found in the DRN and in adjacent portions of the periaqueductal gray matter. There appeared to be no obvious relationship between recording site and responses of neurons to serotonergic agonists and their TPH-immunoreactivity (data not shown).
DISCUSSION

In the present study, electrophysiologically characterized serotonergic and non-serotonergic DRN neurons were defined by the presence and absence of tryptophan hydroxylase immunoreactivity, respectively, as we have done previously in the rostral ventromedial medulla (Marinelli et al. 2002). The primary conclusion of this study is that serotonergic and non-serotonergic DRN neurons are both heterogeneous with respect to their responses to 5-HT and to their action potential characteristics. In addition, many serotonergic and non-serotonergic DRN neurons respond to both 5-HT1A and 5-HT2A/C receptor subtype activation. Thus, the net effect of 5-HT in many DRN neurons is likely to due to a complex balance of 5-HT1A and 5-HT2A/C receptor activation.

Numerous studies have indirectly identified putative serotonergic DRN neurons using a number of pharmacological and electrophysiological criteria (e.g. Aghajanian and Lakoski 1984; Sprouse and Aghajanian 1987; Hajos et al. 1996; Liu et al. 2000; Craven et al. 2001; Haj-Dahmane 2001). These criteria include inhibition (hyperpolarization) by serotonin via 5-HT1A receptor activation and broad action potentials (Aghajanian and Vandermaelen 1982; Xu et al. 1998). By exclusion, non-serotonergic DRN neurons are presumably unaffected, or excited (depolarized) by serotonin via 5-HT2A/C receptor activation and have narrow action potentials. Recent studies examining directly identified serotonergic and non-serotonergic DRN neurons indicate that these neuronal groups are likely to be pharmacologically and electrophysiologically heterogeneous (Kirby et al. 2003; Beck et al. 2004).

In the present study we examined the responses of neurochemically identified serotonergic and non-serotonergic DRN neurons to 5-HT, 5-HT receptor subtype activation and compared their action potential characteristics. Immunocytochemistry for TPH was used to identify serotonergic and non-serotonergic neurons. When using whole-cell recordings, 5-HT appears to become dialyzed out of the cell, making it difficult to reliably identify serotonergic neurons that have been physiologically characterized. In contrast, we have previously found that TPH labeling remains robust following whole-cell recording (Marinelli et al. 2002). However, two caveats should be considered when
interpreting these data. Firstly, the antibody used in these studies may cross-react with the enzyme tyrosine hydroxylase, which synthesizes catecholamines. Since dopaminergic tyrosine-hydroxylase-immunoreactive neurons have been reported in the rostral portion of the DRN (Hokfelt et al. 1976), it is possible that some neurons in these studies were dopaminergic. Only one neuron was recorded in the rostral portion of the DRN in the present study (e.g., at the Bregma – 5.60 mm level (Paxinos and Watson 1998)), which was excluded from subsequent analysis. Thus it is likely that most or all of the TPH-labeled cells included in the present study were serotonergic. Secondly, our identification of TPH-negative neurons relies on negative evidence: a lack of staining. As such, it is possible that some neurons were misclassified and that some of the cells identified as TPH-negative were actually serotonergic. Although possible, this appears unlikely. Filling cells with biocytin did not alter the intensity of their labeling: there was no significant difference between the intensities of biocytin-filled TPH neurons and randomly selected unfilled TPH neurons in the same slices (p > 0.05, n=10 filled TPH cells). Moreover, TPH-ir was frequently strong in the same optical sections as filled, unlabeled neurons were found (see, for instance, Figures 1Biii, 2Biii). In addition, both TPH-positive and TPH-negative neurons were sometimes filled within a single brain slice.

We first characterized neurochemically defined DRN neurons by their responses to 5-HT. As in previous studies, DRN neurons responded to 5-HT with an outward (inhibitory) current, or an inward (excitatory) current. It was found that a greater proportion of both TPH-positive and TPH-negative neurons responded to 5-HT with an outward current. Furthermore, there was a substantial overlap in the magnitude of 5-HT induced currents in TPH-positive and TPH-negative neurons. These observations suggest that 5-HT responsiveness does not exclusively distinguish between serotonergic and non-serotonergic DRN neurons.

We next examined the responses of neurochemically defined DRN neurons to selective activation of 5-HT receptor subtypes. In agreement with prior studies, some of which have used more selective 5-HT receptor ligands, the 5-HT-induced outward and inward currents observed in the present were likely to be mediated by 5-HT1A and 5-HT2A/C receptors, respectively (Xu et al. 1998; Liu et al. 2000; Craven et al. 2001; Kirby et al. 2003; Beck et al. 2004). Virtually all TPH-positive and TPH-
negative neurons responded to 5-HT1A receptor activation (using 5-CT), as observed previously for 5-HT immuno-positive and negative DRN neurons (Kirby et al. 2003; Beck et al. 2004). In addition, a significant proportion of TPH-positive (47%) and TPH-negative (75%) neurons also responded to 5-HT2A/C receptor activation (using DOI). Interestingly, a significant proportion of TPH-positive (31%) and TPH-negative (75%) neurons responded to both 5-CT and DOI; and these neurons responded to 5-HT with either outward (57%), or inward currents (43%). These observations suggest that the response to 5-HT1A and 5-HT2A/C receptor activation does not exclusively distinguish between serotonergic and non-serotonergic DRN neurons. In addition, the net effect of 5-HT in many serotonergic and non-serotonergic DRN neurons is likely to be due to a balance of functional 5-HT1A and 5-HT2A/C receptors, rather than the exclusive presence of one receptor subtype, as observed in prior studies on neurochemically unidentified DRN neurons (Craven et al. 2001).

We next examined the electrophysiological characteristics of neurochemically defined DRN neurons. TPH-positive and TPH-negative neurons did not differ statistically in their membrane capacitance and in the majority of their action potential characteristics. While TPH-positive neurons had on average longer duration action potentials than TPH-negative neurons, there was a substantial overlap. While a longer action potential width has been used to distinguish serotonergic DRN neurons (Vandermaelen and Aghajanian 1983), the present observations are consistent with recent studies which have not reported a consistent relationship between action potential width and 5-HT immunoreactivity (Kirby et al. 2003; Beck et al. 2004). Thus, in conjunction with recent studies, the present observations suggest that action potential width does not exclusively distinguish between serotonergic and non-serotonergic DRN neurons.

The above observations suggest that none of the individual pharmacological, or electrophysiological characteristics measured in the present study absolutely distinguish between serotonergic and non-serotonergic DRN neurons. However, in agreement with previous studies (Aghajanian and Vandermaelen 1982; Vandermaelen and Aghajanian 1983), all neurons which were inhibited by 5-HT (outward current) and had long duration action potentials (> 2.5 ms) were serotonergic (45% of neurons examined). In addition, 86% of neurons which were excited by 5-HT (inward current) and had
short duration action potentials (< 2.0 ms) were non-serotonergic (21% of neurons examined). This suggests that a combination of pharmacological (5-HT response) and electrophysiological (action potential width) characteristics might distinguish at least a subpopulation of serotonergic and non-serotonergic DRN neurons, although this requires further study. It must also be emphasized that a substantial population of DRN neurons (34%) did not fit into either of these pharmacological/electrophysiological categories and were neurochemically heterogeneous.

The source of pharmacological and electrophysiological heterogeneity in serotonergic and non-serotonergic DRN neurons remains unclear. DRN neurons can also be classified according to other characteristics such as differences in other neurotransmitters, e.g. GABA (Belin et al. 1983; Harandi et al. 1987; Charara and Parent 1998). In addition, the DRN contains neurons which project to a wide variety of brain regions and receives inputs from within the DRN itself and also from other nuclei (Halliday et al. 1995). These, or other characteristics might provide additional means to identify serotonergic and non-serotonergic DRN neurons. Our findings suggest that 5-HT is likely to have a complex effect on serotonergic and non-serotonergic neurons in the dorsal raphe nucleus and that prior models of serotonergic actions within the DRN need to be revised. In particular, both serotonergic and non-serotonergic DRN neurons respond to 5-HT with responses ranging from inhibition to excitation, with the net effect of 5-HT relying on a balance of functional 5-HT1A and 5-HT2A/C receptors. Thus, the activity of the different DRN neuronal populations will change in an intricate manner when the serotonergic tone in the DRN is altered by neurological disorders or by drug treatment (e.g., SSRIs or hallucinogens).

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Figure Legends

Figure 1. Serotonergic and non-serotonergic dorsal raphe neurons respond to 5-HT with an outward current. Electrophysiological characterization of two DRN neurons which responded to 5-HT with an outward current and either (A) expressed TPH-ir, or (B) did not express TPH-ir. (Ai) and (Bi) show the current traces for each of these neurons during superfusion of 5-HT (30 µM), DOI (3 µM), 5-CT (100 nM), ketanserin (Ket, 3 µM) and pindolol (Pind, 1 µM) when voltage clamped at –60 mV. (Aii) and (Bii) show the action potential traces for each of these neurons obtained in current clamp mode. (Aiii) and (Biii) show separate images of biocytin labeling of the filled cell (left panel), TPH-ir (middle panel) and a merged confocal image of the biocytin (in red) and TPH-ir (in green) (right panel). Scale bars in (Ai) and (Bi) are 20 pA and 30 s; in (Aii) and (Bii) are 20 mV and 5 ms; and in (Aiii) and (Biii) are 20 µm. In (Aii) the * denotes the presence of an inflection on the repolarising phase of the action potential, and the dashed lines shows where the action potential duration and slow AHP amplitude were measured.
Figure 2. Serotonergic and non-serotonergic dorsal raphe neurons respond to 5-HT with an inward current. Electrophysiological characterization of two DRN neurons which responded to 5-HT with inward currents and either (A) expressed TPH-ir, or (B) did not express TPH-ir. Layout for figure 2 is the same as that for figure 1: showing (i) current traces during superfusion of 5-HT (30 µM), DOI (3 µM), 5-CT (100 nM), ketanserin (Ket, 3 µM) and pindolol (Pind, 1 µM), (ii) action potential traces and (iii) biocytin labeling and TPH-ir of the respective neurons. Scale bars in (Ai) and (Bi) are 20 pA and 60 s; in (Aii) and (Bii) are 20 mV and 5 ms; and in (Aiii) and (Biii) are 20 µm. In (Aii) and (Bii) the * denotes the presence of a fast-AHP and the dashed lines shows where the action potential duration and fast AHP amplitude were measured.
Figure 3. TPH-immunoreactivity in dorsal raphe neurons which respond to 5-HT with outward and inward currents. (A) Bar chart displaying the percentage of TPH-positive neurons (TPH+ve) and TPH-negative neurons (TPH-ve) which responded to 5-HT with an outward (5HT Out, open bar), or an inward current (5HT In, filled bar). (B) Scatter plot displaying the currents produced by 5-HT in TPH-positive and TPH-negative neurons which responded to 5-HT with outward (open circle), or inward currents (filled circle). In (A), the absolute number of responding neurons is shown in each bar. In (B), the horizontal lines represent the mean current.
Figure 4. TPH-immunoreactivity in dorsal raphe neurons which respond to 5-CT and DOI. (A) Bar chart displaying the percentage of TPH-positive neurons (TPH+ve) and TPH-negative neurons (TPH-ve) which responded to 5-CT (open bar), or DOI (filled bar) out of those that were tested. (B) Scatter plot displaying the currents produced by 5-CT (open circle), or DOI (filled circle) in TPH-positive and TPH-negative neurons (only 5-CT and DOI responding neurons are represented). (C) Bar chart displaying the percentage of TPH-positive and TPH-negative neurons which responded only to 5-CT (open bar), only to DOI (black bar), or to both 5-CT and DOI (grey bar). In (A) and (C), the absolute number of responding neurons is shown in each bar. In (B) the horizontal lines represent the mean current produced by 5-CT and DOI in these neurons.
Figure 5. TPH-immunoreactivity and action potential width of DRN neurons. Scatter plot of the action potential durations (AP width) of TPH-positive neurons (TPH+ve) and TPH-negative (TPH-ve) neurons. The horizontal lines represent the mean action potential duration of TPH-positive and negative neurons.
Marinelli et al Figure 5

The figure shows a scatter plot with the x-axis labeled "TPH +ve" and "TPH -ve" and the y-axis labeled "AP Width (ms)". The plot includes data points indicating different AP widths for positive and negative TPH values.