Serotonergic and Nonserotonergic Dorsal Raphe Neurons Are Pharmacologically and Electrophysiologically Heterogeneous

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Marinelli, Silvia, Stephen A. Schnell, Stephen P. Hack, MacDonald J. Christie, Martin W. Wessendorf, and Christopher W. Vaughan. Serotonergic and nonserotonergic dorsal raphe neurons are pharmacologically and electrophysiologically heterogeneous. J Neurophysiol 92: 3532–3537, 2004. First published July 14, 2004; doi:10.1152/jn.00437.2004. 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 nonserotonergic neurons of the DRN, their postsynaptic responses to 5-hydroxytryptamine (5-HT, serotonin) and 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 nonserotonergic neurons responded to 5-HT with outward (i.e., inhibitory) and inward (i.e., excitatory) currents, responded to both 5-HT1A and 5-HT2A/C receptor activation with outward and inward currents, respectively, and displayed overlapping action potential characteristics. These findings suggest that serotonergic and nonserotonergic 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.

INTRODUCTION

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

Directly identified 5-HT-containing DRN neurons have been characterized electrophysiologically as having broad action potentials and low firing rates and being inhibited (hyperpolarized) by 5-HT via 5-HT1A receptor activation (Aghajanian and Vandermaelen 1982; Xu et al. 1998). By exclusion, putative nonserotonergic 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 Laks 1984; Craven et al. 2001; Haj-Dahmane 2001; Hajas et al. 1996; Liu et al. 2000; Sprouse and Aghajanian 1987).

Interestingly, there is evidence that the activity of putative serotonergic and nonserotonergic DRN neurons may be modulated by both 5-HT1A and 5-HT2A/C postsynaptic receptors (Craven et al. 2001; Liu et al. 2000). Furthermore, recent studies have demonstrated that directly identified serotonergic and nonserotonergic DRN neurons both respond to 5-HT1A receptor agonists (Beck et al. 2004; Kirby et al. 2003). However, neurochemically defined serotonergic and nonserotonergic 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 nonserotonergic.

METHODS

Fifteen- to 22-day-old Sprague-Dawley rats were anesthetized with halothane and 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) containing (in mM) 126 NaCl, 2.5 KCl, 1.4 NaH2PO4, 1.2 MgCl2, 2.4 CaCl2, 11 glucose, and 25 NaHCO3. Slices were maintained at 34°C in a submerged chamber containing ACSF equilibrated with a mixture of 5% CO2-95% O2. The brain slices were then transferred to a chamber and superfused continuously (1.8 ml/min) with ACSF (34°C).

DRNs 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 (in mM) 115 K-glucuronate, 25 KCl; 15 NaCl, 1 MgCl2, 10 HEPES, 11 EGTA, 2 MgATP, and 0.25 NaGTP and 0.01% biocytin, pH 7.3, osmolarity 280–285 mosm/L. Series resistance (<20 MΩ) was compensated by 80% and continuously monitored during experiments with an Axo-
patch 200B amplifier (Axon Instruments, Foster City, CA). Postsynaptic currents (under voltage clamp, holding potential: −60 mV, with a liquid junction potential correction of −10 mV) were filtered [100-Hz low pass filter (LPF)] and sampled (500 Hz) for later analysis (Axograph 4, Axon Instruments). Action potentials recorded under current clamp were filtered (20 kHz LPF) and sampled (50 kHz) for analysis. The action potential duration was measured from the threshold of the rapid polarizing phase to an equivalent voltage on the repolarizing phase (see horizontal dashed lines in Figs. 1, Aii, and 2, Aii). The presence of an inflection/hump on the repolarizing phase of the action potential was assessed by visual inspection (see Fig. 1Aii, asterisk) and confirmed by the presence of an inflection on the differentiated action potential trace. The presence of a fast afterhyperpolarization (fast AHP) immediately after the action potential, which was distinct to a longer lasting slow AHP was visually assessed (see Fig. 2, Aii and Bii, asterisk). The amplitude of the fast and slow AHPs were measured from the threshold of the rapid polarizing phase (see Figs. 1, Aii, and 2, Aii and Bii, vertical and horizontal dashed lines).

Biocytin, 5-carboxamidotryptamine maleate (SCT), R(−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) and

![Image](http://jn.physiology.org/)

**FIG. 1.** Serotonergic and nonserotonergic dorsal raphe nucleus (DRN) neurons respond to serotonin (5-HT) with an outward current. Electrophysiological characterization of 2 dorsal raphe nucleus neurons which responded to 5-HT with an outward current and either expressed (A) or did not express tryptophan hydroxylase immunoreactivity (TPH-ir). Ai and Bi: the current traces for each of these neurons during superfusion of 5-HT (30 μM), R(−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI), 3 μM, 5-carboxamidotryptamine maleate (SCT, 100 μM), 3-[2-[4-(4-fluorobenzoyl)-0-piperidinyl][ethyl]2,4-[1H,3H]-quinazolinedione tartrate (ketanserin, 3 μM), and 1-(1H-indol-4-ylox)-3-[(1-nethylethyl)amino]-2-propanol (pindolol) when voltage clamped at −60 mV. Aii and Bii: the action potential traces for each of these neurons obtained in current-clamp mode. Aiii and Biii: separate images of biocytin labeling of the filled cell (left), TPH-ir (middle), and a merged confocal image of the biocytin (in red) and TPH-ir (in green; right). Scale bars in Ai and Bi are 20 μA and 30 s; in Aii and Bii are 20 nA and 5 ms; and in Aiii and Biii are 20 μm. In Aii, * denotes the presence of an inflection on the repolarizing phase of the action potential; , where the action potential duration and slow afterhyperpolarization (AHP) amplitude were measured.

5-HT were obtained from Sigma (Sydney, Australia). 3-[2-[4-(4-fluorobenzoyl)-0-piperidinyl][ethyl]2,4-[1H,3H]-quinazolinedione tartrate (ketanserin) and 1-(1H-indol-4-ylox)-3-[(1-nethylethyl)amino]-2-propanol (pindolol) 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 one to two neurons per brain slice, and recordings from each neuron lasted <20 min. After recording, the slices that containing biocytin-filled cells were fixed for 30–60 min in a phosphate-buffered paraformaldehyde/picric acid solution [75 mM KH₂PO₄; 85 mM Na₂HPO₄; 4% (wt/vol) paraformaldehyde; 14% (vol/vol) saturated aqueous picric acid; pH 6.9]. The slices were then washed six to eight times and stored in a phosphate-buffered sucrose solution [30 mM KH₂PO₄; 70 mM Na₂HPO₄; 10% sucrose (wt/vol); 0.01% (wt/vol) sodium azide; 0.032% (wt/vol) bacitracin; pH 7.2]. Brain stem 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). Tryptophan hydroxylase immunoreactivity (TPH-ir) was visualized using a sheep anti-TPH antiserum (Chemicon, Temecula, CA) 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 ± SE, and statistical comparisons were made using χ² tests for differences among proportions or
unpaired t-test 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 DRNs voltage clamped at ~60 mV. Superfusion of 5-HT (30 μM) produced an outward current in 77% of DRN neurons tested (Fig. 1, A and B, mean current = 26 ± 2 pA, n = 123). 5-HT produced an inward current in 20% of DRN neurons (Fig. 2, A and 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, whereas 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 1-(2-methoxyphenyl)-4-(4 phthalimido-buty)-piperazine (NAN-190, 100 nM, n = 5). Both the 5-HT1A/B/D/7 agonist 5-CT (100 nM, n = 80/89) and the 5-HT1A/7 agonist (±)-8-hydroxy-2-dipropylaminotetralin (8-hydroxy-2-DPAT, 300 nM, n = 6/6) produced outward currents in most DRN neurons that were abolished by co-application of either pindolol (1–3 μM, n = 46; Figs. 1A and 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 DOPI 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; Figs. 1A and 2). These observations are consistent with prior studies that 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 (Beck et al. 2004; Craven et al. 2001; Kirby et al. 2003; Liu et al. 2000; Sprouse and Aghajanian 1987; Xu et al. 1998).

In some of the preceding experiments, the action potential characteristics of DRN neurons were studied in current-clamp mode (Figs. 1, ii, and 2, ii, 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). Fifty percent of neurons displayed an inflection on the repolarizing phase (n = 39). Sixty-four percent of neurons displayed a fast afterhyperpolarization (AHP) after 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 that had a mean amplitude of 24 ± 1 ms (range: 14–38 mV).

TPH immunoreactivity and serotonergic responses of DRN neurons

Following the preceding recordings, 81 biocytin-filled DRN neurons were recovered and examined for TPH immunoreactivity (THR-ir), a marker of serotonergic neurons. Of the recovered biocytin-filled DRN neurons 72% were immunoreactive for TPH (n = 58/81). Both TPH-positive and -negative DRN neurons responded to 5-HT with outward and inward currents (Figs. 1 and 2), although TPH-positive and -negative neurons differed in their proportions of 5-HT outward and inward currents (χ² = 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 (Fig. 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 (Fig. 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 (Fig. 3B).

We next examined the TPH-ir of neurons based on their responses to 5-CT and DOI. The proportion of 5-CT and DOI responders was not significantly different between TPH-positive and -negative neurons (χ² = 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 (Fig. 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 (Fig. 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 that 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 that responded to DOI.

In some of these experiments, we examined the effect of both 5-CT and DOI (Fig. 4C, n = 17). Sixty-two percent (n = 8) of the TPH-negative neurons and 25% (n = 1) of the TPH-negative neurons responded exclusively to 5-CT. Eight percent (n = 1) of the TPH-positive neurons and none of the TPH-negative neurons responded exclusively to DOI. Thirty-one
We next examined TPH-ir in DRN neurons that 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-ir (data not shown).

**DISCUSSION**

In the present study, electrophysiologically characterized serotonergic and nonserotonergic DRN neurons were defined by the presence and absence of TPH-ir, 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 nonserotonergic DRN neurons are both heterogeneous with respect to their responses to 5-HT and to their action potential characteristics. In addition, many serotonergic and nonserotonergic 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; Craven et al. 2001; Haj-Dahmane 2001; Hajas et al.

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We also examined the TPH-ir of neurons based on 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 (Fig. 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 repolarizing phase (χ² = 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 (χ² = 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 that was of similar amplitude (P > 0.05). In addition to action potential characteristics, we also examined the TPH-ir of neurons based on 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).
1996; Liu et al. 2000; Sprouse and Aghajanian 1987). These criteria include inclusion (hyperpolarization) by serotonin via 5-HT1A receptor activation and broad action potentials (Aghajanian and Vandermaelen 1982; Xu et al. 1998). By exclusion, nonserotonergic 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 nonserotonergic DRN neurons indicate that these neuronal groups are likely to be pharmacologically and electrophysiologically heterogeneous (Beck et al. 2004; Kirby et al. 2003).

In the present study, we examined the responses of neurochemically identified serotonergic and nonserotonergic DRN neurons to 5-HT and 5-HT receptor subtype activation and compared their action potential characteristics. Immunocytochemistry for TPH was used to identify serotonergic and nonserotonergic 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 after whole cell recording (Marinelli et al. 2002). However, two caveats should be considered when interpreting these data. First the antibody used in these studies may cross-react with the enzyme tyrosine hydroxylase, which synthesizes catecholamines. Because 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. Second, 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, Figs. 1Biii and 2Biii). In addition, both TPH-positive and -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 -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 -negative neurons. These observations suggest that 5-HT responsiveness does not exclusively distinguish between serotonergic and nonserotonergic 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 (Beck et al. 2004; Craven et al. 2001; Kirby et al. 2003; Liu et al. 2000; Xu et al. 1998). Virtually all TPH-positive and -negative neurons responded to 5-HT1A receptor activation (using 5-CT) as observed previously for 5-HT immunopositive and -negative DRN neurons (Beck et al. 2004; Kirby et al. 2003). 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 nonserotonergic DRN neurons. In addition, the net effect of 5-HT in many serotonergic and nonserotonergic 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 -negative neurons did not differ statistically in their membrane capacitance and in the majority of their action potential characteristics. Although TPH-positive neurons had on average longer duration action potentials than TPH-negative neurons, there was a substantial overlap. Although 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 that have not reported a consistent relationship between action potential width and 5-HT immunoreactivity (Beck et al. 2004; Kirby et al. 2003). Thus in conjunction with recent studies, the present observations suggest that action potential width does not exclusively distinguish between serotonergic and nonserotonergic DRN neurons.

The preceding observations suggest that none of the individual pharmacological or electrophysiological characteristics measured in the present study absolutely distinguish between serotonergic and nonserotonergic DRN neurons. However, in agreement with previous studies (Aghajanian and Vandermaelen 1982; Vandermaelen and Aghajanian 1983), all neurons that 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 that were excited by 5-HT (inward current) and had short-duration action potentials (<2.0 ms) were nonserotonergic (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 nonserotonergic 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 nonserotonergic DRN neu-
rons 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; Charara and Parent 1998; Harandi et al. 1987). In addition, the DRN contains neurons that 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 nonserotonergic DRN neurons. Our findings suggest that 5-HT is likely to have a complex effect on serotonergic and nonserotonergic neurons in the DRN and that prior models of serotonergic actions within the DRN need to be revised. In particular, both serotonergic and nonserotonergic 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., selective serotonin reuptake inhibitors or hallucinogens).

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