Transient Firing of Dorsal Raphe Neurons Encodes Diverse and Specific Sensory, Motor, and Reward Events

Sachin P. Ranade1 and Zachary F. Mainen1,2

1Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; and 2Champalimaud Neuroscience Programme, Instituto Gulbenkian de Ciência, Oeiras, Portugal

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Ranade SP, Mainen ZF. Transient firing of dorsal raphe neurons encodes diverse and specific sensory, motor, and reward events. J Neurophysiol 102: 3026–3037, 2009. First published August 26, 2009; doi:10.1152/jn.00507.2009. Serotonin (5-hydroxytryptamine [5-HT]) is known to influence a wide range of behaviors and physiological processes, but relatively little is known about events that trigger 5-HT release. To address this issue, we recorded from neurons in the dorsal raphe nucleus (DRN) in rats performing an odor-guided spatial decision task. A large fraction of DRN neurons showed transient firing time locked to behavioral events on timescales as little as 20 ms. DRN transients were sometimes correlated with reward parameters, but also encoded specific sensorimotor events, including stimulus identity and response direction. These behavioral correlates were diverse but showed no apparent relationship with waveform or other firing properties indicative of neurochemical identity. These results suggest that the 5-HT system does not encode a unitary signal and that it will broadcast specific information to the forebrain with speed and precision sufficient not only to modulate but also to dynamically sculpt ongoing information processing.

INTRODUCTION

The 5-hydroxytryptamine (5-HT, serotonin) system is one of the most important targets for treatment of depression, anxiety, panic disorder, chronic pain, and other psychiatric conditions. Thus there has been great interest in elucidating its functions and a large number of pharmacological, as well as genetic, experiments have been performed. These data have implicated 5-HT in modulating an extremely wide range of phenomena, leading to a highly diverse set of behavioral and physiological hypotheses of 5-HT function, including behavioral suppression (Soubrie 1986), coordinating defense (Deakin and Graeff 1991), aversive learning (Daw et al. 2002; Dayan and Huys 2008; Deakin and Graeff 1991), motor facilitation (Jacobs and Fornal 1997), carbon dioxide sensing (Richerson 2004), temporal discounting (Doya 2002), and regulating energy balance (Tecott 2007).

The dorsal raphe nuclei (DRN) are located in the midbrain and control the release of most 5-HT in the neocortex (Jacobs and Azmitia 1992). Electrophysiological recordings of DRN neurons may help to elucidate the function of the 5-HT system by determining the conditions that trigger the firing of these cells and consequent 5-HT release. The first recordings in anesthetized animals revealed a strong correlation with behavioral state, with DRN firing being minimal during rapid eye movement sleep and maximal during waking (McIntyre and Harper 1976; Trulson and Jacobs 1979). Although these remain the most widely documented correlates of DRN firing (Urbain et al. 2006), many physiological variables are also correlated with arousal state and might account for the observed relationships. Moreover, an extensive series of studies in awake cats found that the firing rates of putative 5-HT neurons are unaffected by a host of manipulations that would be expected to engage various physiological and behavioral responses, including thermoregulatory, cardiovascular, and glucoregulatory challenges, as well as stress from white noise, restraint or predator exposure, and treadmill locomotion (Jacobs and Fornal 1997; Veasey et al. 1997).

Although these studies primarily focused on longer timescale DRN modulation, consistent with the classical view of neuromodulatory systems as tonically acting signals, relatively less attention has been focused on transients. If 5-HT neurons signal specific behavioral or cognitive variables (Cools et al. 2008), as has been proposed for the dopamine and norepinephrine systems (Aston-Jones and Cohen 2005; Schultz et al. 1997), then transient signals should be particularly revealing (Schultz 2006). It was observed in early studies that DRN neurons respond rapidly and transiently to peripheral nerve stimulation (Aghajanian et al. 1978), consistent with an averse signal. A recent study in primates (Nakamura et al. 2008), using a decision-making task, demonstrated DRN neuron firing is modulated both positively and negatively by predicted and received reward magnitude. These results support the involvement of 5-HT in reward processing, but did not reveal a unitary signal as observed for dopamine neurons (Schultz 2007). These studies also demonstrate the importance of appropriate behavioral paradigms to rigorously test neuronal correlates.

Transient responses of DRN neurons to sensory stimuli including flashes, sound clicks, and light touch (Fornal et al. 1996; Heym et al. 1982; Montagne-Clavel et al. 1995; Waterhouse et al. 2004), as well as relatively specific motor-related responses (Kulichenko and Pavlenko 2004; Veasey et al. 1995) have been reported, suggesting that the conception of DRN transients relating only to reward is narrow. However, the nature of transient DRN sensory and motor responses has not been extensively documented, particularly since the low firing rates of neurons requires a structured behavioral paradigm to characterize.

In the present study, we recorded DRN activity in freely moving rats performing a decision-making task. The use of a two-alternative choice discrimination paradigm provided sensory, motor, and reward components and allowed us to correlate neuronal activity across a large number of repeated trials with a variety of behavioral variables with precise timing. We
report that a large majority of DRN neurons are rapidly and transiently modulated by sensory and motor as well as reward variables. These responses were more diverse and selective than previously appreciated, particularly for sensory and motor variables. These results demonstrate that the DRN processes a wide range of information, suggesting that transient 5-HT signals from the DRN will broadcast surprisingly detailed information to the neocortex.

METHODS

Animal subjects

Male Long–Evans rats weighing 250–350 g were used for all experiments. Rats had free access to food but water was restricted to behavioral session and about 30 additional minutes in their home cage such that weights were maintained within 85% of their free feeding weight. All procedures involving animals were carried out in accordance with National Institutes of Health standards as approved by the Cold Spring Harbor Laboratory Institutional Animal Care and Use Committee.

Two-odor discrimination task

All behavioral procedures were conducted as previously described in Uchida and Mainen (2003). Briefly, behavioral setup consisted of a box with a panel containing the three ports equipped with infrared photodiode and phototransistor. Interruption of the infrared photo beam signaled time of entry of rat into the port. Odors were mixed with pure air to produce a 1:10 dilution at a flow rate of 1 l/min using a custom-built olfactometer. Delivery of odors and water reinforcement were controlled using computer data acquisition hardware (National Instruments, Austin, TX) and custom software written in MATLAB.

Rats were trained and tested on a two-alternative choice odor-discrimination task as follows. Rats initiated a trial by poking their nose into the central odor-sampling port, which triggered delivery of one of two odors after a random delay of 0.3–0.5 s. After waiting at the odor-sampling port for a period of ≥0.1 s, the rat was free to respond by withdrawing from the odor port and moving to the left or right choice ports. Each odor signaled availability of water in one of the two choice ports. Odors were used individually or in a bundle) tetrodes. Tetrodes consisted of four twisted 17-μm platinum–iridium wires (Neuralynx, Tuscon, AZ). Tetrodes were advanced slowly through steps of 40 μm and the DRN was identified as the area following a region of low electrical noise as the tetrodes passed through the ventricle. Electrode tracks were visualized by coating a fluorescent marker, 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes, Eugene, OR), near the tip of the tetrode. The final position of each track was marked by making an electrical lesion (Fig. 1C).

Analysis

CV2. Variability of spike train was measured using a metric CV2, a measure of spike train variability that is not sensitive either to mean firing rate or to slow changes in firing rate. CV2 is the average measure of the variance of two adjacent interspike intervals (ISI). CV2 is lower for regular spike trains and higher for irregular spike trains (Holt et al. 1996).

DEFINITION OF BEHAVIORAL EPOCHS. The task-engaged state (T) was the time spent by rat performing the task. It was defined as the time interval containing at least one port entry every 2 s. The sleep state (S) was defined from hippocampal local field potential (LFP) and the cortical EEG as follows. Sleep bouts were identified by increased power in lower frequency (delta-theta band) and decrease in power in the gamma band (Fig. 1E). The awake state (A) was defined as the time when the rat was neither sleeping nor performing the task. The task modulation index (MT) was calculated as the ratio of difference in firing rate between task-engaged and awake state to the sum of the firing rates during the two epochs: $MT = (f_T - f_S) / (f_T + f_S)$. A sleep modulation index (MS) was calculated similarly. The modulation indices have a range between −1 and 1, with 0 indicating no change, negative values indicating suppression of firing with respect to awake firing rate, and positive values indicating enhancement.

RECEIVER OPERATOR CHARACTERISTIC (ROC) ANALYSIS. Due to relatively low firing rates, we used a nonparametric method from signal detection theory, ROC analysis, to index the modulation of neuronal firing by different task parameters (Green and Swets 1966). ROC-based measures have been extensively used for comparing and quantifying neuronal responses to stimuli and to provide a quantitative estimate of how well the neuronal response is able to classify the stimulus (Britten et al. 1992). The area under the ROC curve, $ROC_{area}(A, B)$, measures the ability of an ideal observer to assign on
FIG. 1. Neurons in the dorsal raphe nucleus (DRN) exhibit state dependent modulation. A: schematic diagram of recording strategy. Cartoon of coronal section of rat brain at the level of the DRN with attached guide cannula and microdrive with tetrodes. Guide cannula was inserted into the brain and tetrodes were gradually lowered through the cannula to the DRN. B: inset in A is magnified. Shaded gray area shows extent of the DRN (DR) and recording area. aq, cerebral aqueduct; pag, periaqueductal gray. C: histological verification of tetrode track. Coronal section of the rat brain with a single tetrode track passing through the DRN observed by fluorescence imaging of DiI (red). D: extracellular spike waveforms of 52 neurons recorded from DRN of 7 rats. Neurons in orange are classified as wide spiking (WS) neurons and narrow spiking (NS) neurons are in blue (Supplemental Fig. S1A). Neuron marked with black circle is an example of a “classical” WS putative 5-HT neuron. Numbers next to waveform indicate neuron ID. E: state-dependent modulation of firing rate of WS neuron marked in D. Top: spectrogram of hippocampal local field potential (LFP). Middle: timing of task events (black line) and classification into Sleep (blue), Task Engaged (green), and Awake (red) behavioral states. Bottom: the binned firing rate for the neuron. Firing rate of the neuron is lowered during sleep. F: average firing rates during the 3 behavioral states for neuron in E. Firing rate decreases during sleep compared with the awake state and remains unchanged during the task state. G: sleep index is plotted as a function of spike width. Most neurons show suppression of firing during sleep. There is no significant correlation between sleep index and spike width. Black line is the best-fit line and \( R^2 \) is the coefficient of correlation. Black circle denotes neuron shown in D–F. H: task index is plotted as a function of spike width.
FIG. 2. Firing patterns of DRN neurons during 2-odor discrimination task. A: schematic diagram of the timing of behavioral events during an example trial. Timing of behavioral events is recorded using the interruption of the infrared photobeam inside the ports (odor and water ports) and odor and water are delivered via computer-controlled valves. An example of all major events during a correct trial is shown. B: normalized firing rates for all recorded neurons aligned to all behavioral events during the task. Pseudocolor plot shows peri-event firing rate modulation of 52 neurons recorded during the task, with firing rates scaled from zero to maximum firing rate during the task for each neuron. Neurons are sorted by increasing average firing rate during the Task epoch. Numbers to the left indicate neuron IDs and correspond to Fig. 1D. Neuron IDs in red were excluded from subsequent analyses because of low overall firing rates. Neuron with asterisk is the same as in Fig. 1D. Black line segments at the bottom show fixed 300-ms peri-event behavioral epochs. C: number of neurons showing significant receiver operating characteristic (ROC) modulation index (RMI, \( P < 0.01 \)) during 5 behavioral epochs depicted in B. Inhibitory responses (red) and excitatory responses (green) are shown separately. D: many DRN neurons modulate their firing rate during more than one epoch. Bar graph shows number of neurons as a function of number of epochs that show significant firing rate modulation. Epochs are the same as in B and C. E and F: strongest transient modulation during behavioral epochs is not correlated with state-dependent modulation during sleep (E) and width of spike waveform (F). Black circle marks WS neuron shown in Fig. 1 and in B. Color code is identical to Fig. 1D. Black line is the best-fit line and \( R^2 \) is the coefficient of correlation.
a trial-by-trial basis a signal to two distributions, A and B. ROC_{AREA} ranges from 0 (when values of A are all larger than any value of B) to 1 (when the opposite holds), with 0.5 indicating indistinguishable distributions. For further details, see Feierstein et al. (2006). The ROC_{AREA} index is preferable to simple measures of normalized firing rate differences because it is independent of assumptions about the nature of the underlying distributions and is not susceptible to noise resulting from very low firing rates. For convenience, we report two indices calculated by scaling the ROC_{AREA} between −1 and 1, so that 0 reflects no difference in distributions.

ROC MODULATION INDEX (RMI). To compare firing rate modulation during a behavioral epoch with respect to baseline firing rate, we defined an ROC modulation index, \( RMI = 2[ROC_{AREA}(f_E - f_B) - 0.5] \), where \( f_E \) is the distribution of firing rates across trials for epoch \( E \) and \( f_B \) is the distribution of firing rates across trials for the baseline epoch \( B \). Thus if distribution of firing rates during an epoch was lower than baseline, RMI has negative values (suppression), whereas an increase in firing rate generates positive RMI values (enhancement).

PREFERENCE INDEX. To compare two trial conditions within the same epoch, we defined a preference index as in Feierstein et al. (2006): \( \text{PREF}_{AB} = 2[ROC_{AREA}(f_A - f_B) - 0.5] \), where \( f_A \) and \( f_B \) are the firing rate distributions for trials of each of two conditions (e.g., odors A and B; movement directions, left and right). For this index, +1 indicates a preference for one odor or movement condition, whereas −1 indicates the opposite preference.

Statistical significance of RMI and preference index values was determined using a permutation test (Feierstein et al. 2006). We recalculated index values after randomly reassigning all firing rates to either of the two groups, repeated this procedure 1,000 times to obtain a distribution of ROC values, and calculated the fraction of values exceeding the actual ROC value. The \( P \) value calculated using this method is equivalent to the nonparametric Wilcoxon test (Hanley and McNeil 1982). For all analyses, we tested for significance at \( P = 0.01 \).

Time windows (epochs) for analysis of firing rates were aligned with respect to specific behavioral events. Figure 2B shows all epoch windows used in the analysis. The baseline distribution was formed by randomly selecting \( n \) time intervals of 300 ms from periods during the awake (nontask) state, where \( n \) was chosen to match the number of trials contributing to the behavioral epoch firing rate distribution. Trials in which a second behavioral event occurred within the analysis window were excluded. An optimal window size of 300 ms was determined to enable inclusion of the maximum number of trials. Only units with \( \geq 10 \) trials per condition were included.

Peristimulus time histograms (PSTHs) were binned with a time window of 15 ms and smoothed with a Gaussian filter of SD 30 ms.

Z-SCORE. For sliding z-score analysis shown in Fig. 7B, each time bin in the smooth PSTH was transformed to a z-score by subtracting from it the mean and dividing by the SD for the entire PSTH for the neuron. Neurons were sorted by their average z-score value during the odor-sampling period for plotting in Fig. 7B.

RESULTS

We recorded the activity of 52 neurons in the DRN of seven freely behaving rats. Recording electrodes (tetrodes) were targeted to DRN using a guide cannula (Waterhouse et al. 2004) to obtain access through the midline sinus (Fig. 1A; see METHODS). Electrode tracks were recovered using standard histological methods at the termination of the experiment (Fig. 1C). In the subsequent analyses, all neurons recorded within the anatomical boundaries of the DRN (Fig. 1, A and B, shaded area) were included.

Firing properties of DRN neurons are heterogeneous

Previous recording studies have identified putative 5-HT neurons on the basis of a wide action potential waveform and metronomic, low-frequency firing (Aghajanian and Vandermaelen 1982; Trulson and Jacobs 1979). However, recent studies indicate that this is neither a necessary nor a sufficient criterion (Allers and Sharp 2003; Hajos et al. 2007; Urbain et al. 2006). In our study, DRN neurons showed heterogeneous
extracellular waveforms (Fig. 1D). We classified DRN units with spike width >1.2 ms as wide spiking (WS) and the remainder as narrow spiking (NS) (Fig. 1D, Supplemental Fig. S1B; WS, orange; NS, blue).\(^1\) Spike width was defined as the time from peak of depolarization and return to baseline after the hyperpolarization phase (Supplemental Fig. S1A). Average firing rates of DRN neurons ranged from 0.01 to 28 spikes/s with a mean of 3.3 spikes/s and showed a wide range of firing regularity, as measured by CV2 (see METHODS), ranging from 0.25 to 1.40 with a mean of 0.85. Neither mean firing rate nor firing variability correlated with spike width (Supplemental Fig. S1, C and D).

A hallmark of putative 5-HT neurons from classical electrophysiological recordings is slow, tonic modulation of firing rate across the sleep–wake–arousal cycle (Trulson and Jacobs 1979). We examined firing rates of DRN neurons during three behavioral states: sleep, task-engaged, and awake but not task-engaged. These states were identified by analyzing behavioral activity and hippocampal LFP (Fig. 1, E and F; see METHODS). The strength of state-dependent modulation was quantified by calculating a normalized index of the change in average firing rate relative to the awake, non-task-engaged state (see METHODS). DRN neurons showed a range of sleep and task modulation, with an overall trend for suppression of firing rate during sleep (Fig. 1G; mean sleep index = 0.81 ± 0.32) and enhancement of firing rate in the task-engaged state (Fig. 1H; mean task index = 1.24 ± 0.58). There was no significant correlation between spike width and sleep modulation (Fig. 1G; \(R^2 = 0.002\), N.S.) or task modulation (Fig. 1H; \(R^2 = 0.014\), N.S.). We also examined the relationship between other firing properties—firing rate and CV2—with state-dependent rate modulation. There was no significant correlation between the firing rate of a neuron and degree of state-dependent modulation (\(R^2 = 0.0003\), N.S. for sleep index and \(R^2 = 0.006\), N.S. for task index; Supplemental Fig. S2, A and C). There was no correlation between the variability of ISI as measured by CV2 and sleep index (\(R^2 = 0.037\), N.S.; Supplemental Fig. S2B), although there was a significant positive correlation with task index (\(R^2 = 0.162, P < 0.01\); Supplemental Fig. S2D). Thus task-modulated neurons tend to be more bursty than average.

**DRN neurons exhibit diverse firing patterns time-locked to task events**

To examine how DRN neurons respond to behavioral events, we correlated neuronal firing with events during the performance of a two-alternative choice odor-discrimination task (Uchida and Mainen 2003). In this task, the rat sampled the odor stimulus at the center port and responded by moving to one of two choice ports located on either side, where water was delivered for correct responses. The timing of entry into and exit from the three ports (center odor port and left and right water ports) was monitored using infrared phototransistor/light-emitting diode pairs, enabling us to correlate DRN firing with locomotor activity in relationship to port entry and exits. In addition, the timing of odor and water delivery was controlled by computer-actuated solenoid valves, enabling us to correlate DRN firing with stimulus and reward delivery (Fig. 2A).

Most DRN neurons responded during the task with changes in firing rate. Rats performed 255 ± 190 trials per session, allowing us to detect firing rate modulation even in neurons with low firing rates. Figure 2B shows normalized PSTHs as pseudocolor maps for all recorded neurons time-locked to all six behavioral events. DRN neurons show a diverse set of responses, encompassing a range of patterns. Many DRN responses were very transient and tightly locked to specific task events (e.g., Fig. 2B; neurons 23 and 30). To quantify these patterns of firing-rate modulation, we compared the firing rates in different behavioral epochs (defined as 300-ms fixed time windows relative to specific behavioral events; Fig. 2B, bottom) to the baseline firing rate using a receiver operator characteristic (ROC)–based metric that we refer to as the ROC modulation index (RMI). RMI ranges from −1 to 1, with negative values denoting suppression of firing and positive values enhancement (see METHODS). Eleven of 52 neurons were excluded from these analyses due to very low firing rates during the task (Fig. 2B, neuron numbers indicated in red).

**TABLE 1. Percentage of DRN neurons modulated during selective task actions**

<table>
<thead>
<tr>
<th>Type of Modulation</th>
<th>Enhancement</th>
<th>Suppression</th>
<th>Total</th>
<th>Percentage of Modulated Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Modulation of firing rates during port approach</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odor port approach only</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>12.2</td>
</tr>
<tr>
<td>Water port approach only</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>12.2</td>
</tr>
<tr>
<td>Both odor and water approach</td>
<td>14</td>
<td>1</td>
<td>15</td>
<td>36.6</td>
</tr>
<tr>
<td>Opposite tuning at both ports</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>9.7</td>
</tr>
<tr>
<td>No approach-related modulation</td>
<td>—</td>
<td>—</td>
<td>12</td>
<td>29.3</td>
</tr>
<tr>
<td>Total number of neurons</td>
<td>21</td>
<td>8</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td><strong>B. Modulation of firing rates during port withdrawal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odor port withdrawal only</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>20.0</td>
</tr>
<tr>
<td>Water port withdrawal only</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>Both odor and water withdrawal</td>
<td>10</td>
<td>2</td>
<td>12</td>
<td>30.0</td>
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<tr>
<td>Opposite tuning at both ports</td>
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<td>3</td>
<td>6</td>
<td>15.0</td>
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<tr>
<td>No withdrawal-related modulation</td>
<td>—</td>
<td>—</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>Total number of neurons</td>
<td>21</td>
<td>8</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

This table shows the percentages of neurons significantly modulated (\(P < 0.01\)) either during port approach or during port withdrawal. Also included are actual numbers showing suppression or enhancement.
As a population, approximately equal numbers of DRN neurons responded during each different epoch of a behavioral trial. Modulation during most epochs was characterized by an increase in firing rate, with the exception of the reward epoch, in which approximately half of the modulated neurons were suppressed (Fig. 2C). Most neurons responded to two or more events (Fig. 2D; e.g., Fig. 2B: 16 and 37), although some responded to only one (e.g., Fig. 2B: 5 and 47), whereas a small fraction of neurons appeared to be completely insensitive to task events, showing extremely steady, clocklike firing (~10%, 4 of 41; e.g., Fig. 2B: 5 and 47). There was no apparent correlation between the extent of state-dependent modulation and strength of transient modulation ($R^2 = 0.004$, N.S.; Fig. 2E). Furthermore, neither spike width ($R^2 = 0.055$, N.S.; Fig. 2F) nor ISI variability ($R^2 = 0.036$, N.S.; Supplemental Fig. S3B) showed any correlation with the strength of transient modulation, as discussed further in the following text. There was a weak but significant positive correlation between firing rate and strength of modulation ($R^2 = 0.25; P < 0.01$; Supplemental Fig. S3A). Thus DRN neurons showed a high degree of heterogeneity of firing properties and exhibited substantial transient modulation time-locked to behavioral events. In the next sections, we will examine in detail the behavioral correlates of raphe neurons during the different phases of the task.

**Locomotor correlates of DRN firing are context dependent**

A large fraction of neurons (83%; 34 of 41) showed significant firing rate modulation (measured using RMI, at a criterion of $P < 0.01$; see Methods) during either entry or exit from the odor and/or choice ports. To determine whether these responses were more closely related to general movement/locomotion or, alternatively, whether they might reflect other variables, we compared the responses of neurons during approach to and withdrawal from the ports. Approach and withdrawal both involve motor “activation” but differ in not only the specific muscle groups involved but also the context and goal of the action. Of the 34 modulated neurons, 10 (29%) neurons had similar modulation at both ports (i.e., odor or choice) and entry or exit, suggesting a general locomotor correlate (Fig. 3A). Most of these neurons also responded to other task events; e.g., the neuron in Fig. 3A also shows click-evoked response (subsequently discussed). The remaining two thirds of neurons (71%, 24 of 34) were modulated selectively during specific actions (i.e., entry or exit), at specific ports, or a combination of both. Figure 3C shows the example of a neuron that fired only in response to exit from the choice ports but not from the odor port. A particularly prominent type of selective modulation was an increase in firing as the animal approached the odor port to initiate the trial (43%, 18 of 42). Although a majority of these neurons also responded during water port entry, a few neurons (22%, 4 of 18) showed a ramp in firing exclusively during odor port entry (Fig. 3B). The proportions of neurons with selective locomotor correlates are summarized separately for port approach and port withdrawal (Table 1).

In addition to locomotion, the task sequence also involved periods of motor suppression when the rat held its snout in either the odor port awaiting the odor stimulus or the water port awaiting water delivery. Although the mechanics of movement inhibition was very similar at the two types of ports, inhibition of firing was often restricted to only the odor port (43%, 3 of 7 neurons; Fig. 3D) or water port (50%, 4 of 8 neurons). Thus suppression of firing during nose pokes also appeared to be specifically related to task requirement rather than to holding, waiting, or movement cessation per se. The specificity of responses suggests that DRN neurons do not simply respond to gross motor components of the task but instead, or in addition, to the context in which those movements are executed.

**FIG. 4. Specificity of tuning of raphe neurons.** A: example of an odor-selective neuron. The 2 odors are air mixtures of enantiomers of 2-octanol. Rasters and PSTH are aligned to onset of odor presentation. Trials in raster are sorted by correct response. Trials in raster are sorted by direction of movement and sorted by duration of odor sampling (blue ticks indicate time of exit from odor port). Color of sidebar indicates trials in which $S+%$-2-octanol (blue) or $R-$-2-octanol (green) were presented. This neuron was activated by $S+%$-2-octanol. The black bar above raster indicates fixed 300-ms time window used for ROC analysis. B: example of a direction-selective neuron. Raster and PSTH are aligned on exit from odor port. Trials are grouped by direction of movement and sorted by duration of movement to the water port (blue ticks indicate time of entry into water port). Only trials with correct responses are included. Trials with movement to left port are indicated by golden sidebar, whereas movement to right port is indicated by purple sidebar. This neuron responds to leftward movement. C: population histogram of odor preference index. Preference index was calculated using ROC analysis (see Methods) during odor sampling epoch indicated in A to quantify selectivity of firing to one of the 2 odors. Colored bars indicate significant selectivity with $P < 0.01$ based on a permutation procedure: blue, cells selective for $S+%$-2-octanol; green, cells selective for $R-$-2-octanol; gray bars, not significant. Asterisk indicates preference value for example neuron shown in A. D: population histogram of direction preference. Direction (left/right) selectivity was calculated during movement epoch indicated in B. Colored bars indicate significant direction preference ($P < 0.01$): violet, selective for movement in rightward direction; golden, selective for leftward direction; gray, not significant ($P < 0.01$). Asterisk indicates preference value for example neuron shown in B.
DRN neurons are tuned to specific odor stimuli and movement directions

The preceding analysis points toward substantial specificity of DRN responses. To further this analysis, we examined firing in relation to specific trial types. That is, during each trial one of two different odors is presented and is rewarded for selection of one of two choice ports, yielding four trial types (two stimuli × two responses). Several DRN neurons preferentially responded to presentation of one of the two odor stimuli (Fig. 4A). We quantified this tuning specificity by defining a preference metric scaled between 0 and 1, where 0 indicates no difference in firing rate for the two odors, negative values indicate preference for odor A [S-(+)-2-octanol], and positive values indicate preference.

![Diagram of odor port, odor valve, water port, water valve](image)

**FIG. 5.** Water valve click responses. A: example of click-responsive neuron. Raster–PSTH is aligned to water valve onset (blue ticks represent water valve switch-off time). B: click vs. tone responses. Latency of the peak of the sound-evoked response is plotted against strength of the response. Neurons tested with clicks are plotted in blue, whereas those tested with pure tone are plotted in red. Pure tone did not evoke strong, short-latency responses as evoked by the click. Neuron shown in A is marked by an asterisk.

**FIG. 6.** Reward correlates of raphe neurons. A and B: neuronal responses are correlated with reward variables. Spikes are aligned to entry into water port. Trials with correct responses are grouped by water delivery (blue) or omission (pink). Trials are sorted by the duration of stay in the water port. Blue ticks indicate time of exit from the water port. Both trial types were randomly interleaved in the session. Distribution of reward delays for the behavioral session is shown as a box plot, with the red line denoting the median and the box denoting the interquartile range. A: example neuron encoding reward delay. This neuron shows a monotonic decline in firing rate on entry into water port. Firing rate reaches its minimum around the time of water delivery. This neuron also responds to the offset of water delivery in the reward-delivered trials (blue). B: example neuron responding to reward omission. This neuron shows an increase in firing rate for trials where water was omitted (pink). Increase in firing rate precedes exit from the water port (blue ticks). C: a small subset of neurons responds to reward omission. Top: sliding ROC preference analysis was performed on 50-ms time windows to compare firing rates between correct trials where water was delivered with those where water was omitted. Time intervals with significant preference values are plotted in pseudocolor. Red intervals indicate increased firing during water delivery ("reward preference"), whereas blue intervals indicate increased firing during water-omission trials ("omission preference"). Neurons shown in A and B and Supplemental Fig. S5 (cells 20 and 26) are indicated on the right. Bottom: number of neurons at each time interval that prefer delivery or omission. Omission preference neurons are represented in blue, reward preference neurons in red. A small subset of neurons shows significant omission preference that emerges after the time of expected reward.
for odor B [R(-)-2-octanol] (Feierstein et al. 2006). Of the 28 neurons that showed significant RMI during odor sampling, 10 neurons showed odor-selective firing as indicated by significant preference values ($P < 0.01$, using a permutation procedure; see METHODS) (Fig. 4C).

During each trial the rat responded by movement to either the left or the right choice port to obtain water. Of the 31 neurons that showed significant modulation during movement to the choice port, 9 (29%) were selective (by calculation of preference at $P < 0.01$) for one of the two directions of movement (Fig. 4, B and D). These results demonstrate a strong specificity of tuning of DRN neurons to alternative stimuli or actions. Only 2 of 17 odor- or direction-selective neurons maintained selectivity during both epochs.

**Prominence of transient responses to solenoid valve click**

A large fraction of DRN neurons showed firing-rate modulation during the reward phase of the task (Fig. 2C). The predominant modulation during this behavioral epoch was a transient response triggered by the solenoid valve that was activated to deliver water (Fig. 5A). Around half of the neurons tested with the solenoid click (43%, 10/23 neurons), showed a significant click-evoked response ($P < 0.01$). These responses were rapid, with latencies as low as 20 ms, and transient, with duration typically <200 ms (Fig. 5, A–C and Supplemental Fig. S4A). In some cases, the response consisted of a single spike without apparent change in the overall firing rate (Fig. 5A and Supplemental Fig. S4B). To further characterize the properties of these click-responsive neurons, in a subset of sessions ($n = 18$ neurons), we silenced the solenoid valve by placing it outside the recording chamber and substituted it by a pure tone (8 kHz) delivered coincident with water port entry. The pure tone predicted water as faithfully as the click of the valve but was different in its acoustic properties, being narrow-band as opposed to the broadband click stimulus, which had a higher initial sound intensity. Moreover, during initial stages of training, rats often show a startle response to the sound of the water valve, but they never did so in response to the tone. Two of eighteen neurons tested with pure tone showed a significant phasic tone-evoked response ($P < 0.01$). The relative proportions of click- versus tone-responsive neurons were significantly different (two-tailed Fisher’s exact test, $P < 0.05$), peak latencies of tone responses were relatively long, and absolute changes in firing rates for click response were significantly higher than those for tone responses (Mann–Whitney U test, $P < 0.01$; Fig. 5B).

**Reward timing is reflected in DRN firing**

Delivery of water was delayed by several different schedules across different recording sessions (see METHODS). The delay schedules included both fixed (1 s) and random (exponential with mean of 0.82 s) distributions and helped to facilitate identification of reward responses and to allow us to check for a relationship of firing to reward timing. In addition, water was omitted on a small subset (20%) of correct responses, a relationship of firing to reward timing. In addition, water was delivered. This procedure allowed us to test for correlates of reward anticipation as well as omission.

In a small subset of neurons, the time course of modulation of firing rate coincided very well with the average delay to the onset of water delivery. Figure 6A shows a neuron that showed a decrease in firing rate that accumulated until the time of expected reward. Similar behavior was observed in four neurons. To test for correlates of reward omission, we compared firing-rate distributions in correct trials, in which water was delivered, with trials in which water was omitted using a sliding ROC analysis (see METHODS). Note that fewer neurons were eligible for this analysis because we required a minimum number of 10 correct reward-omitted trials. Interestingly, a small subset of neurons (12% or 4/32; preference at $P < 0.01$) responded with a change in firing rate during omission trials around the expected time of reward (Fig. 6, B and C and Supplemental Fig. S5, A and B). It is important to note that no overt sensory signal occurs at the time of reward omission. Also note that the omission responses are correlated with differences in movement that occur not during but subsequent to reward omission.

As noted earlier (Fig. 2D), many neurons responded during more than two epochs within the task. Our data set did not allow for quantitative analysis of all combinations of observed responses; however, one response profile was particularly prominent. A large proportion of click-responsive neurons also

![FIG. 7. Multiplicity of responses of DRN neurons. A: example of neuron showing odor port inhibition, movement excitation, and water valve click response. Left panel is aligned to entry into odor port; middle panel aligned to exit from the odor port; and right panel aligned to water valve onset. Only correct trials are shown. B: z-score analysis. Normalized z-scores (see METHODS) plotted for all neurons recorded with an audible water valve click. Each row is a single neuron; z-scores of firing rates are plotted in pseudocolor. Panel alignments are identical to those in A. Neurons are ordered by their average z-score values during the odor-sampling epoch. Neuron shown in A is indicated by an asterisk. Many neurons that are inhibited during odor sampling are also excited during movement and show a phasic click-evoked response. Color bar: z-score.](http://jn.physiology.org/doi/abs/10.1152/jn.00856.2009)
exhibited suppression of firing during odor sampling and an increase in firing during movement to water port (Fig. 7, A and B). Conversely, neurons that were inhibited while waiting at the water port were often excited during odor sampling (Fig. 7B).

Responses of DRN neurons do not correlate with waveform properties

We have shown that rat DRN neurons respond to diverse sensory, motor, and reward-related events. We examined whether the task-related responses showed any correlation with spike characteristics indicative of serotonergic and nonserotonergic neurons. Neither spike width (Fig. 8) nor firing rate and firing variability (Supplemental Fig. S6) were correlated with firing-rate modulation during any of the task epochs. Furthermore, the response characteristics of the best candidate “classical” 5-HT neurons (wide spike, low firing rate, sleep suppression) spanned the entire range of observed behavioral correlates, including odor selectivity, valve-click response (Fig. 5A), odor-port–specific inhibition, reward timing, and reward omission. Thus we found no evidence that putative serotonergic neurons or nonserotonergic DRN neurons display a special class of behavioral correlates.

DISCUSSION

We recorded from the DRN in rats performing a decision-making task. The large majority (70%) of DRN neurons was rapidly modulated by behavioral events on time scales of 200 ms and as short as 20 ms. DRN firing was highly heteroge-

TABLE 2. Responses of midline and lateral wing DRN neurons

<table>
<thead>
<tr>
<th>Type of Modulation</th>
<th>Midline (n = 8)</th>
<th>Lateral (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor port inhibition</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Odor response</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Movement response</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Odor selectivity</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Direction selectivity</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Click response</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>

This table compares a set of responses of midline and lateral subdivision DRN neurons. There was no significant dependence between anatomical location and presence or absence of response, as calculated by two-tailed Fisher’s exact test at $P < 0.05$. 

FIG. 8. Relationship between behavioral correlates and firing properties. A–C: modulation of firing rates during behavior is not correlated with spike width. RMI for all neurons plotted as a function of spike width during 3 behavioral epochs (i.e., odor sampling, movement, and reward). Significant RMI values ($P < 0.01$; METHODS) are shown for enhancement of firing (green) and suppression (red) compared with firing rate during the baseline. Whereas nonsignificant values are shown in gray.
DRN. Eight neurons were from the midline and 15 were from the lateral subdivision. Response profiles for the two groups of neurons are summarized in Table 2. Although we did not see any statistically significant differences between the two subdivisions, our small sample size is not sufficient for a rigorous test of this hypothesis.

DRN responses frequently showed a high degree of sensory specificity. Over one third of DRN neurons that were modulated during odor sampling were selectively tuned to specific odor stimuli (Fig. 4, A and C). To our knowledge, this is the first demonstration that DRN neurons show specific sensory tuning. Sensory selectivity suggests a possible mechanism for stimulus-specific forms of “top-down” modulatory control. For example, the DRN sends a very large projection to the olfactory bulb, where it may modulate early olfactory sensory processing (Pezold et al. 2009; Ranade and Mainen 2004).

More than half of recorded neurons responded to the “click” sound of the solenoid valve used to deliver water with an extremely rapid, transient response, peaking in as little as 20 ms, a latency lower than that previously reported for auditory responses (Heym et al. 1982; Waterhouse et al. 2004) and comparable to that of responses observed in auditory cortex (Heil and Irvine 1997). This indicates that serotonin release may be capable of modulating even the earliest stages of cortical sensory processing. Indeed, the click-evoked transients might be related to suppression of processing of the solenoid noise, consistent with the involvement of 5-HT in the suppression of sensory responsivity (Hurley and Pollak 2001; Kayama et al. 1989; Waterhouse et al. 1990) and startle reflexes (Davis et al. 1980). Postsynaptic ionotropic 5-HT3 receptors with extremely rapid kinetics (Chameau and van Hooft 2006) represent a possible mechanism for rapid readout of such 5-HT transients.

Many DRN neurons responded selectively to specific actions during the task action sequence (Figs. 3 and 4, B and D)—even actions that shared general locomotor characteristics, such as nose pokes into different ports (Fig. 3B), but differed in the details of the motor pattern and context. DRN firing has previously been reported to correlate with locomotion (Fornal et al. 1996; Veasey et al. 1997; Waterhouse et al. 2004) and rhythmic behaviors (Fornal et al. 1996; Veasey et al. 1997). Previous studies have also documented relatively specific motor correlates, e.g., oral–buccal movements associated with feeding but not yawning (Fornal et al. 1996; Veasey et al. 1997), and direction of eye movements (Nakamura et al. 2008). However, these studies showed more tonic modulations (~1 s) and were relatively sparse (Nakamura et al. 2008). The more robust and finer scale of temporal modulation we observed could be explained by the combination of stereotypy resulting from a well-learned behavior sequence (vs. freely behaving cats; Fornal et al. 1996; Veasey et al. 1997) and greater range of motor activity due to the absence of restraint (vs. head-fixed monkeys; Nakamura et al. 2008; cf. Kulichenko and Pavlenko 2004). Specific motor responses may be related to the activation of specific central pattern generators (Hattox et al. 2003; Schmidt and Jordan 2000), possibly on the timescale of individual cycles (Kepecs et al. 2007).

We frequently observed DRN neurons that were activated during both odor port and choice port entry and exit (motor facilitation), but were inhibited during odor sampling (sensory disinhibition and motor inhibition) (Figs. 3 and 7). Moreover, the same DRN neurons that responded to the solenoid valve (discussed earlier) tended to show suppression during odor sampling (Fig. 7). These patterns of activity are reminiscent of the “motor hypothesis” proposed by Jacobs and Fornal (1997). According to this proposal, DRN neuronal firing facilitates motor processes and inhibits sensory processes. Thus periods of increased 5-HT firing are associated with movement and suppression of sensation, whereas decreased 5-HT activity is associated with movement cessation and enhancement of sensation.

In addition to frank sensory and motor responses we also observed a relatively large fraction of neurons responding during the reward phase of the task, as reported previously (Nakamura et al. 2008). Phasic 5-HT signals have been theorized to report a negative error in reward prediction or an unexpected aversive outcome (Daw et al. 2002) and we tested this idea using reward omissions. Indeed, a small subset of neurons (4/32) responded to reward omission around the expected time of reward (Fig. 6). The relative scarcity might be attributable to a failure of the omissions to trigger behavioral adjustments. Interestingly, we also observed several examples of DRN neurons in which the temporal structure of firing rates was matched to the temporal expectation of reward in the absence of direct sensory cue or overt motor responses (Fig. 6A).

The diversity, specificity, and precision of behavioral correlates we report demonstrate that the DRN receives substantial information relating not only to a wide variety of sensory- and motor-related but also to reward-related events and 5-HT neurons may access and broadcast this information. These findings reinforce the challenge of assembling a unity theory of 5-HT function and underline the importance of determining how informational diversity maps onto the anatomical and neurochemical diversity of the DRN and how transient firing of DRN neurons is read out by its widespread downstream targets.

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REFERENCES

Diversity and Specificity of Raphe Transients


