Activity of long-lead burst neurons in pontine reticular formation during head-unrestrained gaze shifts

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Walton MM, Freedman EG. Activity of long-lead burst neurons in pontine reticular formation during head-unrestrained gaze shifts. J Neurophysiol 111: 300–312, 2014. First published October 30, 2013; doi:10.1152/jn.00841.2012.—Primates explore a visual scene through a succession of saccades. Much of what is known about the neural circuitry that generates these movements has come from neurophysiological studies using subjects with their heads restrained. Horizontal saccades and the horizontal components of oblique saccades are associated with high-frequency bursts of spikes in medium-lead burst neurons (MLBs) and long-lead burst neurons (LLBNs) in the paramedian pontine reticular formation. For LLBNs, the high-frequency burst is preceded by a low-frequency prelude that begins 12–150 ms before saccade onset. In terms of the lead time between the onset of prelude activity and saccade onset, the anatomical projections, and the movement field characteristics, LLBNs are a heterogeneous group of neurons. Whether this heterogeneity is endemic of multiple functional subclasses is an open question. One possibility is that some may carry signals related to head movement. We recorded from LLBNs while monkeys performed head-unrestrained gaze shifts, during which the kinematics of the eye and head components were dissociable. Many cells had peak firing rates that never exceeded 200 spikes/s for gaze shifts of any vector. The activity of these low-frequency cells often persisted beyond the end of the gaze shift and was usually related to head-movement kinematics. A subset was tested during head-unrestrained pursuit and showed clear modulation in the absence of saccades. These “low-frequency” cells were intermingled with MLBs and traditional LLBNs and may represent a separate functional class carrying signals related to head movement.

FOVEATE SPECIES CAN OBTAIN high-resolution information about a visual scene only through the use of a series of gaze shifts. When the head does not contribute to these movements, saccadic eye movements are highly stereotyped (Bahill et al. 1975; Bahill and Stark 1977; Baloh et al. 1975a, b; Becker 1989). Neurophysiological studies in awake, behaving primates have demonstrated impressive correlations between the kinematic characteristics of head-restrained saccades and measures of neural activity (Cullen and Guitton 1997a; Hepp and Henn 1983; Kaneko et al. 1981; Ling et al. 1999; Scudder et al. 1988). Anatomical studies have shown that firing rate is predictive of eye velocity (Kaneko et al. 1981; Keller 1974; Luschei and Fuchs 1972; van Gisbergen et al. 1981). When the head is free to move, however, the fit for some inhibitory-burst neurons can be improved by adding a head-velocity term (Cullen and Guitton 1997b).

Long-lead burst neurons (LLBNs) are a group of cells that are believed to relay signals from the superior colliculus (SC) to MLBs in PPRF and the rostral interstitial nucleus of the medial longitudinal fasciculus (Hepp and Henn 1983; Keller et al. 2000). In general, LLBNs are characterized by low-frequency prelude activity that begins anywhere from ~12 to ~150 ms before the onset of ipsiversive saccades. Some have been reported to have movement fields similar to those of saccade-related neurons in SC (Hepp and Henn 1983). Others exhibit a monotonic increase in peak firing rate associated with increases in the peak velocity; a similar monotonic relationship has been reported between the number of spikes in the burst and the amplitude of the horizontal component of the saccade (Hepp and Henn 1983; Scudder et al. 1988). Anatomical studies have demonstrated direct projections to MLBs, as well as extrinsic projections to nucleus reticularis tegmenti pontis (Keller et al. 2000; Moschovakis et al. 1996; Scudder et al. 1996a, b).

This variability in tuning characteristics and anatomical connectivity suggests the possibility that more than one functional class may exist within the population of neurons that is currently referred to as LLBNs collectively. The use of head-restrained subjects may not be ideal for the identification of functional distinctions, however, because of the stereotyped nature of these movements and the tight coupling of kinematic variables. For example, amplitude, peak velocity, and duration are intercorrelated.

When the head is free to move, it is possible to dissociate many of these kinematic variables. When the initial positions of the eyes in the orbits are varied, gaze shifts of a particular vector can be associated with a wide range of eye amplitudes, peak velocities, and durations; the eye amplitude-duration relationship, for example, can have a negative slope (Freedman 2008). The freedom of head movement also makes it possible to investigate the possibility that some PPRF neurons might carry signals related to head movement. Data consistent with this hypothesis arise from experiments showing that stimulation of PPRF in head-unrestrained monkeys often evokes movement of both the eyes and the head (Gandhi et al. 2008).

The present study characterizes the activity of LLBNs when the head is free to move, and gaze, eye, and head kinematics are dissociable. We used a behavioral task that permitted the...
experimental control of the initial positions of the eyes in the orbits. We describe the activity of a large population of neurons in PPRF that is characterized by low firing rates, prelude activity, and the continuation of discharge beyond the end of the gaze shift. We compare and contrast the discharge properties of these cells with those of classically defined LLBNs.

**METHODS**

Four rhesus monkeys (*Macaca mulatta*) served as subjects. Two sterile surgeries were performed to prepare each animal for neurophysiological experiments. In the first, a stainless-steel post was affixed to the skull with bone screws, and a teflon-coated coil of wire was implanted under the conjunctiva of one eye (Judge et al. 1980). Following this, the monkeys were trained on the required behavioral tasks (see below). When they became proficient at these tasks, a second surgery was performed to affix a recording chamber over a trephine craniotomy. The chamber was positioned on the midline at stereotaxic zero (*monkeys U, S, and P*) or 1 mm caudal to zero (*monkey Q*). All surgical and experimental procedures were approved by the University of Rochester Animal Care and Use Committee and were in compliance with the National Institutes of Health Guide for the Care and Use of Animals.

During experiments, animals sat in a custom-designed primate chair that restricted movements of the torso but allowed the head to move without restriction. Before each experiment, a small, lightweight cam-lock device was fitted to the head post. This device held three red (650 nm) diode lasers and a teflon-coated coil of wire, similar to the one implanted on the eye. This coil was used to measure head position. The central laser was directed along the midsagittal plane of the subject; the other two were aimed 18° away from the midsagittal plane—one to the right and one to the left. Before recording began each day, the head was released, but the cam-lock device remained attached to the head. This allowed the animal to move its head freely in any direction. During data collection, the monkey chair sat in the center of a cube that housed three pairs of magnetic-field coils (CNC Engineering, Seattle, WA). The current generated in the eye and head coils was linearly related to the horizontal rotational position of the coil within ~2% over 360°. Signals from the gaze and head coils were sampled at 1 kHz and filtered using a five-pole low-pass Bessel filter with a cutoff frequency of 3 kHz. A second low-pass filter (time constant = 0.3 ms) was used to condition coil signals before digitizing. All aspects of the experiment were controlled through the use of custom software running on a personal computer with an extended peripheral component interconnect bus (National Instruments, Austin, TX). For most experiments, targets were presented on a flat light-emitting diode (LED) board that spanned ±48° horizontally and ±40° vertically and contained LEDs every 2°. On some days, however, targets were presented on the inside surface of a 1.5-m-diameter hemisphere (0.5 in acrylic; Capital Plastics, Beltsville, MD) using two lasers attached to independent, two-axis-motorized gimbals (RGV 100 rotation stages; Newport, Irvine, CA). These gimbals position visual targets with better than 0.01° accuracy.

Single neurons in PPRF were isolated using tungsten microelectrodes (Micro Probe, Gaithersburg, MD) and were amplified (Bak Electronics, Mount Airy, MD), filtered, and saved for offline analysis. During data collection, PPRF was identified by the presence of high-frequency burst activity associated with ipsiversive gaze shifts and by the proximity of the recording site to other known neurophysiological landmarks, such as omnipause neurons, SC, trochlear nucleus, and abducens motoneurons. When neurophysiological recording was completed, marking lesions were made in both hemispheres in one animal. The tracks were run near the caudal edge of where MLBs had been encountered. The electrode was advanced until we could no longer hear the characteristic, robust background bursting associated with ipsiversive gaze shifts. The electrode was then retracted 0.5 mm. Marking lesions were then made by passing 50 μA direct current for 30 s. Additional marking lesions were made for each millimeter as the electrode was retracted 5 mm. The animal was then killed. The brain was cut into 50 μm slices and stained with cresyl violet. In addition to recording the digitized spike times, the analog signal from the electrode was digitized at 25 kHz and used offline to confirm the isolation of each neuron.

**Behavioral Tasks**

Two tasks were used.

*Delay task.* Figure 1A shows a schematic representation of this task. At the start of each trial, a green visual target (T1) was illuminated. At the same time, a randomly selected, head-mounted laser was switched on. The monkeys were then required to look to and align the laser spot with this visual target within 500 ms. When the center laser was used, this required the eyes to be approximately centered in the orbits. The use of the left or right laser required the eyes to be directed to the left or right in the orbits, respectively. If the animal maintained the alignment of both gaze and the laser spot successfully with this target for a variable period (500–1,000 ms in 100-ms increments), a second visual target (T2) was switched on at a different location. Monkeys were then required to keep the eyes and laser spot directed at the first target for an additional period (the “delay period”; 300–600 ms in 50-ms increments). The end of the delay period was signaled by the switching off of the head-mounted laser and the fixation target. The animal was then permitted to use any combination of eye and head movements to look to the location of the second target. If the monkey maintained fixation on this target for 500–1,000 ms (100-ms increments), a reward was given.

*Target blank task.* This task was used to study postgaze neural activity in the absence of corrective saccades. It was identical to the delay task except that the second target was extinguished during the gaze shift (Fig. 1B). In this case, the animal was rewarded for maintaining fixation at the location of the second target for 500–1,000 ms (100-ms increments), although it was no longer present.

The location of the second target was chosen randomly to elicit gaze shifts with horizontal components, ranging from 10° contralateral to 70° ipsilateral to the recording site. In general, the increments

![Fig. 1. Schematic representation of behavioral tasks. A: in the delay task, a head-mounted laser and a visual target (T1) were illuminated. The monkey was required to look to and align the laser spot with this target. After a variable fixation period, a 2nd target (T2) was switched on. The animal had to maintain fixation at T1 for the delay period. When T1 and the head-mounted laser were switched off, the animal was permitted to look to the location of T2. Successful fixation of this target resulted in a liquid reward. B: the target blank task was identical to the delay task, except that T2 was extinguished shortly after the initiation of a gaze shift to look to it. The reward was contingent on successful fixation of the spatial location of the (now-extinguished) T2.](http://jn.physiology.org/doi/10.1152/jn.00841.2012.supp)
were \(10^5\), with the exception that an additional target was sometimes used to elicit \(4^\circ\) ipsiversive gaze shifts. To identify cells with SC-like movement fields, movement fields were plotted for each cell. For this reason, 50% of the trials required gaze shifts with vertical components of \(10^\circ\), \(20^\circ\), \(30^\circ\), or \(40^\circ\) up or down. On the other 50% of trials, there was no vertical component.

**Data Analysis**

Gaze and head onset and offset times were determined by using a sliding window algorithm (Walton et al. 2007, 2008). “Gaze duration” was always taken to be the time interval between the beginning of the gaze shift and gaze end. “Gaze amplitude” was the change in gaze (eye-in-space) position between gaze onset and gaze offset. “Eye amplitude” was the change in eye position (eye-in-head) between these two time points. The term “head amplitude” will be used to refer to the change in head position between the measured beginning and end of the head movement. Note that this is distinct from “head contribution,” which refers to the change in head position during the gaze shift.

Neural activity was analyzed within a time window that was defined in two different ways. In the first approach, the time window began 150 ms before gaze onset and ended at gaze offset. For the second method, the time of highest firing rate was found. The algorithm then moved backwards in time, until the instantaneous firing rate first dropped below 20 spikes/s. The end of the measuring window was found by sliding the window forward in time until the instantaneous firing rate dropped below 20 spikes/s. Burst lead was defined as the time interval (in milliseconds) between the beginning of the burst (identified using the second approach) and the start of the gaze shift. Similarly, burst lag was defined as the time interval (in milliseconds) between the end of the gaze shift and the end of the burst. Negative values of burst lag indicate that the burst ended before the gaze shift did. Cells with leftward-on-directions were highly similar to those with rightward-on-directions, so the data were pooled for purposes of analysis.

If isolation of a given neuron were maintained for at least 50 correctly performed trials, linear regressiveon analyses were performed to test for relationships between the number of spikes in the burst and the horizontal and vertical component (gaze) amplitudes. For this analysis, it was necessary to use a window that would not be influenced by differences in the times of onset of prelude activity and activity that occurred after the end of the gaze shift. For these analyses, therefore, the spikes were counted within a fixed time interval, referenced to gaze onset and offset (see above).

Some cells had relatively low firing rates (peak frequency never exceeded 400 spikes/s for gaze shifts of any vector tested). In fact, a continuum was found between cells with peak spike frequencies in excess of 800 spikes/s and those that never exceeded 100 spikes/s. Rather than assume a priori that neurons with lower firing rates represent a distinct functional class, we proceeded from the assumption that they were all LLBNs. A given neuron was, therefore, considered to be a LLBN if the following criteria were met: 1) the neuron was recorded in or near PPRF (see Fig. 10); 2) there was a clear increase in firing rate associated with at least some gaze shifts; 3) the cell did not meet the Hepp and Henn (1983) criteria for classification as a MLB.

However, to determine whether cells with high firing rates exhibited different properties from lower-frequency cells, the cells in our sample were divided into two groups based on peak firing rate. To this end, all trials with ipsiversive gaze shifts were binned according to horizontal gaze amplitude. All bins were \(10^\circ\) in size, with bin centers of \(10^\circ\), \(20^\circ\), \(30^\circ\), \(40^\circ\), \(50^\circ\), \(60^\circ\), and \(70^\circ\). Within each bin, the mean peak firing rate was computed. For purposes of data analysis, cells were divided into two groups based on the highest mean peak firing rate found for any of these bins. A threshold of 400 spikes/s was chosen arbitrarily. A cell with a mean peak firing rate \(>400\) spikes/s for any bin was considered to be a “higher-frequency cell”; a cell that did not have a mean peak firing rate above that value for any bin was considered to be a “lower-frequency cell.”

To identify cells with SC-like movement fields, the mean number of spikes was computed for each unique horizontal target position. A cell was considered to have a SC-like movement field if a significantly lower number of spikes (two-tailed t-test) were found for the (70,0) (for cells in the right PPRF) or (−70,0) (for cells in the left PPRF) target position than for any target position with a horizontal eye amplitude less than that. When this was the case, the location of the movement field peak was considered to be the target position corresponding to the largest mean number of spikes.

**RESULTS**

A total of 269 neurons met the criteria for basic analyses. Of these, 141 were classified as LLBNs. Of the remaining neurons, 63 were classified as MLBs, 17 as burst-toric cells, and 10 as vertical-burst neurons. The other 38 cells were task-related neurons that did not fit into any of the above categories. Data from the 63 MLBs are described elsewhere (Walton and Freedman 2011). Figure 2 shows three groups of four trials from an example LLBN—one with a high peak firing rate. All trials illustrate rightward gaze shifts along the horizontal meridian, having amplitudes between \(49^\circ\) and \(51^\circ\). Shown are the eyes deviating to the left in the orbits by \(\sim 20^\circ\), the eyes close to the center of the orbits, and the eyes deviating to the right (\(\sim 17^\circ\)). Note that as the initial eye position becomes more rightward (ipsilateral to the direction of the gaze shift), the eye saccade amplitude decreases, and the head contribution increases (Freedman 2005, 2008; Freedman and Sparks 1997). The figure also illustrates eye-, head-, and gaze-velocity traces for these movements. As the head contribution increases, peak eye and gaze velocities decrease, and the duration of the gaze shift increases. Rasters and cumulative spike histograms (bin width = 10 ms) for the example trials are also shown. In the figure, the low-frequency activity begins nearly 100 ms before gaze-shift onset and leads to a high-frequency burst that precedes the beginning of the gaze shift by approximately 15–20 ms. Note that the shape of the burst is a reasonable match for the shape of the gaze- and eye-velocity profiles. The duration of the high-frequency burst closely matches the duration of the gaze shift. Note the absence of spikes after the end of the gaze shift.

Figure 3 shows another example cell. All conventions are the same as those in Fig. 2. The movements shown here are very similar to those shown in the corresponding panels of Fig. 2. An examination of the rasters and cumulative spike histograms reveals that the firing rate is considerably lower for this cell than for the cell shown in Fig. 2. Indeed, this cell shows no sign of a high-frequency burst. Instead, the discharge begins approximately 100–150 ms before gaze-shift onset and leads to a high-frequency burst that precedes the beginning of the gaze shift by approximately 15–20 ms. Note that the shape of the burst is a reasonable match for the shape of the gaze- and eye-velocity profiles. The duration of the high-frequency burst closely matches the duration of the gaze shift. Note the absence of spikes after the end of the gaze shift.

Figure 4 shows movement field plots for the cells shown in Fig. 2 (Fig. 4A) and in Fig. 3 (Fig. 4B). The number of spikes in the burst is shown. For both cells, the number of spikes increases monotonically as the horizontal gaze amplitude increases. However, due to the lower-firing rate, the spike count is considerably lower for the cell shown in Fig. 4B. Based on
an analysis of the number of spikes in the burst (see METHODS), a minority of the cells displayed SC-like movement fields. Six of the higher-frequency cells had movement fields centered at 5°–10° horizontal. Two had movement fields centered at approximately (30,0) and one had a movement field centered near (–20,30). For lower-frequency cells, only one showed a vectorial preference. This cell showed significantly more spikes for gaze shifts near (–20,0). For all other cells, the number of spikes increased monotonically with increasing horizontal gaze amplitude.

Based on the relationships between number of spikes and amplitude, three of the LLBNs were tuned predominantly for vertical gaze shifts. None of these resembled the low-frequency cells that are the subject of this report (i.e., all three had vigorous, high-frequency bursts that were limited to the time of the gaze shift). For all of the remaining 138 cells, the total number of spikes (measured until the firing rate dropped below 20 spikes/s; see METHODS) was better (and significantly) correlated with horizontal gaze and/or head amplitude. It should also be pointed out that the range of possible vertical target locations was limited to ±40°. Over that range, the vertical head movement tends to be rather limited (see Fig. 1 of Freedman 2005), which would make it difficult to perform a robust statistical analysis. This is particularly true for low-frequency cells, which, by definition, have a lower range of possible firing rates. By contrast, we were able to elicit gaze shifts of 70° or

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Fig. 2. Example trials from a long-lead burst neuron (LLBN) with a high peak firing rate. Three groups of 4 trials are shown, with initial eye positions to the left (left column), centered in the orbits (middle column), or to the right (right column). All columns show rightward gaze shifts with amplitudes between 49° and 51°. The 1st row shows eye (black), gaze (blue), and head (red) position traces. The 2nd row shows velocity traces for each. The 3rd and 4th rows show rasters and cumulative spike histograms for this cell. As the initial eye position becomes more rightward, the head contribution increases, the duration increases, and the peak eye and gaze velocities decrease. Note that the cell consistently ceases discharge shortly before the end of the gaze shift.
more along the horizontal dimension. Therefore, as previous studies of LLBNs have done (Cullen and Guitteny 1997a, b), subsequent analyses focus on the horizontal component.

To quantify the highest firing rate for each cell in a way that was independent of the tuning characteristics, the data were binned in 10° bins, according to horizontal gaze amplitude (see METHODS). Figure 5 shows, for each cell, the mean peak firing rate associated with the bin with the highest firing rate. Note the large numbers of cells that never attain firing rates above 300 spikes/s for gaze shifts of any vector within the range of vectors that we elicited. There is a suggestion of bimodality here, but a Hartigan's dip test failed to reveal a significant deviation from a monomodal distribution ($P = 0.74$).

A minority of cells ($n = 10$) displayed very low-frequency activity (<20 spikes/s) during the delay period. Perhaps due to the very low firing rates, we were unable to correlate this activity with any visual- or movement-related parameters, so it will not be discussed further.

**Neural Activity After the End of the Gaze Shift**

One can clearly see that the high-frequency cell shown in Fig. 2 stops firing shortly before the end of the gaze shift, whereas the low-frequency cell shown in Fig. 3 continues to discharge well beyond the end of the gaze shift. To determine whether this is a consistent difference between cells with high vs. low firing rates, we computed the median burst lag (see METHODS) for each cell. The median was used, because for some cells, a small minority of trials with prolonged postgaze activity produced misleading values for the mean. The median burst lag was not correlated significantly with gaze amplitude for any of the cells in our sample. Figure 6A shows a histogram of median burst lag for all cells. The large number of cells that

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Fig. 3. Example trials from a cell with a low peak firing rate. All conventions are the same as Fig. 2. Although the movements are highly similar to the ones shown in Fig 2, this neuron’s discharge is quite different. In addition to the much-lower firing rate, note that the cell consistently continues to discharge after the end of the gaze shift.

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ceases discharge during the 50-ms period preceding gaze shift end clearly stands out from the rest of the distribution. The example cells shown in Figs. 2 and 3 suggest the possibility that cells that cease discharging before gaze shift end might also be the ones with high firing rates. To investigate this, histograms were plotted separately for higher- and lower-frequency cells. As this figure shows, nearly all of the higher-frequency cells ceased discharge during the 50-ms period preceding the offset of the gaze shift. A very different picture can be seen in the histogram of median burst lag for the lower-frequency cells. The majority of lower-frequency cells continued to discharge after the end of the gaze shift, with many continuing for another ~100 ms or more.

These data suggest the possibility that two distinct, functional classes may exist. To determine whether cells with high firing rates have different functional properties than those with low firing rates, an arbitrary frequency cutoff of 400 spikes/s (see Fig. 5) was used to divide LLBNs into two groups (see METHODS). Sixty-four of the LLBNs were classified as high-frequency cells, and 74 were classified as low-frequency cells. The next step was to determine whether the two groups have distinct properties.

As stated above, the postgaze activity seen in many of the lower-frequency cells might also be prelude activity associated with small-amplitude, corrective saccades. We tested this possibility in 33 cells (11 high frequency and 22 lower frequency) by interleaving target blank trials randomly (see METHODS) with delay trials. For all of these cells, the use of target blank trials consistently eliminated the corrective saccades. For the lower-frequency cells, the elimination of corrective saccades did not abolish the postgaze activity. Figure 7 plots the median burst lag on the target blank trials vs. the median burst lag on the delay trials for each of these cells. A given target blank trial was used for this analysis only if there were no detectable saccades in the 400-ms period following the offset of the primary saccade. All lower-frequency cells that showed postgaze activity in the presence of corrective saccades also showed postgaze activity in the absence of corrective saccades.
Dynamic Analysis

To investigate the possibility that the activity of low-frequency cells might be related to head movement, we used a second-order differential equation to predict the firing rate

$$ FR(t - d) = y + g_0 H(t) + g_1 \dot{H}(t) + g_2 \ddot{H}(t) $$

where $FR$ is the firing rate, $d$ is the dynamic lead time (see below), $H$ is head position, letters with overdots are derivatives, $t$ is time, and $g_0$, $g_1$, and $g_2$ are constants. For cells with leftward-preferred directions, the signs were reversed, such that a positive slope always indicates that firing rate increased for higher values of velocity or acceleration. Since the proper dynamic lead time for head cells is not known, the fit was performed using time shifts ranging from 0 to 100 ms (in 5-ms increments). For each cell, the time shift producing the best fit was taken as the dynamic lead time. Since most of the low-frequency cells continued to discharge after the end of the gaze shift, this analysis included the entire head movement. Since this period included both the saccade and the postgaze vestibulo-ocular reflex phase (two very different eye movements), we did not include eye-movement terms in this analysis.

We compared models of different complexities, using a stepwise regression approach. As each term was added, the goodness-of-fit was assessed by computing the root-mean-square error and the coefficient of determination ($R^2$; variance accounted for). To be conservative, a given variable was added to the model only if it reduced the root-mean-square error by at least 10%. Figure 8 shows an example of this fit for one low-frequency cell that showed a head velocity and a bias term. For 40/59 low-frequency cells (68%), the firing rate was positively correlated with at least one head movement-related parameter. For most of these, firing rate was positively correlated with only one variable: head position (two cells, 3%), head velocity (22 cells, 37%), and head acceleration (13 cells,
For two neurons (3%), firing rate was related to head velocity and head acceleration. There was one cell for which firing rate was related to head position and head acceleration. Across all low-frequency cells, the mean slope for head position was \( \frac{1}{1000} 0.19 \) (SD \( \frac{1}{1000} 1.03 \); range \( \frac{1}{1000} 6.87 \) to 1). For head velocity, the mean slope was 0.18 (SD \( \frac{1}{1000} 0.23 \); range \( \frac{1}{1000} 0.14 \) to 1.10). For head acceleration, the mean slope was 0.01 (SD \( \frac{1}{1000} 0.0157 \); range \( \frac{1}{1000} 0.02 \) to 0.08).

For the 38 cells that were related to head velocity and/or acceleration, the mean dynamic lead time was 55.378 ms (49 ± 39.5 ms for the velocity cells; 62 ± 36.4 ms for the acceleration cells). Across all low-frequency cells, the mean \( R^2 \) for these fits was 0.24. Across all cells with velocity-related activity, the mean slope for this parameter was 0.36; for the head-acceleration cells, the mean slope for this parameter was 0.03.

When the same equation was used to fit the data from the high-frequency cells, the results were quite different. None showed significant positive correlations with head velocity, but 17/55 (31%) showed significant negative correlations. Across all high-frequency cells, the mean slope for head position was \( -0.81 \pm 1.66 \) (range = \( -3.99 \) to 4.25). The mean slope for head velocity was \( -0.38 \pm 0.56 \) (range = \( -1.72 \) to 0.63). For head acceleration, the mean slope was 0.017 ± 0.03 (range = \( -0.03 \) to 0.10). However, only four (7%) showed significant positive correlations with head acceleration. None showed negative correlations with head acceleration independent of head velocity.

**Low-Frequency Cell Discharge During Head-Free Pursuit**

If low-frequency cells only carry head-movement signals specifically related to saccadic gaze shifts, then they should
be quiescent during head-unrestrained pursuit. On the other hand, if they carry signals related to movement of the head, then one might expect them to discharge whenever the head is moving, regardless of whether it is associated with a gaze shift or pursuit. We tested this by recording from an additional 10 low-frequency cells during both gaze shifts and pursuit with the head free to move (see METHODS).

Figure 9 shows example data from one cell. Figure 9A shows data for a 1,500-ms period, aligned on the onset of 50° gaze shifts (same format as Fig. 3). The appearance of a burst in the cumulative spike histogram is due to the compressed time scale.

The peak firing rate for this cell never exceeded 350 spikes/s for any gaze vector elicited by our tasks. Note that like most low-frequency neurons, this cell continues to discharge well after the end of the gaze shift. Figure 9B shows four example head-unrestrained pursuit trials aligned on the cue to initiate pursuit and a period of >350 ms, during which the head was moving, but no detectable saccades occurred on any of the four trials. Note that the cell consistently continued to discharge during this period and begins to discharge very early on one of the trials. This early activity coincided with a small, on-direction head movement that was associated with neither a gaze shift nor pursuit.

**Fig. 9.** Example data from a low-frequency cell recorded during both gaze shifts and head-unrestrained pursuit. **A:** the cell showed a consistent increase in firing rate associated with 50° leftward gaze shifts. It continued to discharge well after the end of the gaze shift until approximately the time the head stopped moving (gray, shaded area). **B:** four head-unrestrained pursuit trials. The gray, shaded area shows a period of >350 ms, in which the head was moving, but no detectable saccades occurred on any of the trials. The cell consistently discharged during these periods. Note the 1 trial in which the cell began to discharge in advance of the pursuit movement. This early discharge was observed consistently in association with small, on-direction, “head-only” movements (marked with arrows). **C:** four-example, head-unrestrained pursuit trials, in which the direction of pursuit reversed unpredictably. Trials are aligned on the cue to initiate pursuit and a period of >350 ms, during which the head was moving, but no detectable saccades occurred on any of the four trials. Note that the cell consistently continued to discharge during this period and begins to discharge very early on one of the trials. This early activity coincided with a small, on-direction head movement that was associated with neither a gaze shift nor pursuit.
higher- and lower-frequency cells were often found in close proximity to each other. Often, while recording lower-frequency cells, MLBs and high-frequency LLBNs could be heard in the background. On several occasions, a lower-frequency cell and a MLB or higher-frequency LLBN were recorded at the same site. Thus it appears that lower-frequency cells are also found in PPRF, in close proximity to higher-frequency LLBNs and MLBs.

DISCUSSION

We have characterized the activity of LLBNs in PPRF with the head unrestrained under conditions that permit the dissociation of kinematic variables. Low-frequency cells were intermingled with high-frequency LLBNs and with MLBs. Indeed, on several occasions, MLBs and low-frequency cells were recorded simultaneously. The higher- and lower-frequency cells in our sample appear to have very little in common, however, beyond the fact that both groups discharge in association with gaze shifts, and both groups show prelude activity. Indeed, the firing rates of most low-frequency cells were positively correlated with head-movement kinematics. The firing rates of high-frequency cells, however, were much more likely to be negatively correlated with head-movement kinematics. This likely reflects the fact that peak eye and gaze velocity begin to decrease as gaze amplitude becomes large, whereas head velocity continues to increase monotonically (Freedman and Sparks 2000). Thus the firing rate of a cell that encodes eye or gaze velocity could be negatively correlated with head velocity if the range of gaze amplitudes is sufficiently large. A cell carrying head signals, however, should continue firing after the end of the gaze shift, and the firing rate should be related to at least one head-movement parameter. Due to the more complex musculature and dynamics of head movements, however, one should not expect the kind of machine-like relationships between firing rate and kinematics that we see from MLBs and gaze velocity.

Cells were initially divided into a higher-frequency group and a lower-frequency group based on an arbitrary peak firing-rate cutoff. If the mean peak firing rate never exceeded 400 spikes/s for any bin, then the cell was considered to be a lower-frequency cell. Subsequent data analysis suggests that this is likely to be a useful way to distinguish between higher- and lower-frequency cells. However, as noted previously, there was a continuum of peak firing rate, rather than two completely distinct groups. For this reason, the distinction should probably be based on other factors as well. Lower-frequency cells exhibit the following properties: 1) a prelude of activity that begins 15–200 ms before gaze onset; 2) low peak firing rates (50–400 spikes/s) and the absence of a clearly defined burst; 3) a continuation of discharge after the end of the gaze shift (for most); 4) firing rate is related to the kinematics of the head movement; 5) they show continued activity during extended, saccade-free periods (>300 ms) of head-unrestrained pursuit. Taken together, these observations strongly imply that low-frequency cells represent a distinct functional class and should not be regarded merely as a low-frequency version of LLBNs.

It is worth considering whether the lower-frequency cells described in the present report correspond to the reticulospinal neurons (RSN), described previously in the cat (Grantyn and Berthoz 1987) and monkey (Robinson et al. 1994; Scudder et
al. 1996b). The eye-neck RSN (EN-RSN) reported by Grantyn and Berthoz (1987) generally had relatively low peak firing rates. Also, similar to our lower-frequency cells, their EN-RSN continued to discharge after the end of the gaze shift. However, their recordings were made in head-restrained animals and appeared to consist mostly of saccades with amplitudes $<15^\circ$. The firing rates of their RSN were much greater than those of our lower-frequency cells over that range of amplitudes. Similarly, the two anatomically identified RSN reported by Scudder et al. (1996b) also exhibited relatively robust discharge associated with saccade amplitudes $<10^\circ$. Importantly, our lower-frequency cells showed little or no response for gaze shifts with amplitudes $<15^\circ$. It would be difficult to perform any meaningful analyses on these cells if they had been recorded with the head restrained, because the response is just too weak over that range of amplitudes. It seems likely, in fact, that most of our lower-frequency cells would have been missed entirely if the head had not been free to move. Nonetheless, we cannot rule out the possibility that our lower-frequency cells might be RSN.

Some of the lower-frequency cells displayed characteristics that suggest the possibility that their discharge may be related to head movement. First, the activity of most of these cells continues after the end of the gaze shift (even in the absence of a corrective saccade), during a period when the head is moving, but the gaze shift has ended. Second, the firing rates of most of these cells (see RESULTS) were positively correlated with head velocity and/or acceleration. Third, when we used both gaze and head-unrestrained pursuit tasks, nine of 10 low-frequency cells showed increased firing rates for both. With respect to this last result, we cannot exclude the possibility that these 10 cells carried both pursuit and gaze saccade commands, but the simpler explanation is that some low-frequency cells discharge during both kinds of movement, because they carry signals specifically related to movement of the head. The plausibility of this hypothesis is also supported by data published previously. Microstimulation of most PPRF sites in head-unrestrained monkeys evokes movement of both the eyes and the head (Gandhi et al. 2008). Numerous studies have documented projections from PPRF to the spinal cord in both cats (Grantyn and Berthoz 1987; Iwamoto and Sasaki 1990; Iwamoto et al. 1990) and monkeys (Robinson et al. 1994; Scudder et al. 1996b).

Head movement-related signals could potentially reach PPRF from several sources, including SC (Walton et al. 2007), frontal eye fields (Knight 2012), and central mesencephalic reticular formation (Pathmanathan et al. 2006). Rezvani and Corneil (2008) reported that low-frequency activity in SC correlates with neck-muscle electromyography (EMG) activity. In addition, single neurons have been reported in SC that discharge in association with head movements that occur in the absence of gaze shifts (Walton et al. 2007). Taken in the context of these earlier reports, the present data suggest that eye and head circuits share many of the same areas of the brain, if not necessarily the same neurons. Functionally, this should not be surprising, since the accuracy of large gaze shifts requires control of the relative timing of the eyes and head.

Since SC projects directly to PPRF, it is worth discussing the present results in light of Walton et al. (2007). Their low-frequency cells in SC clearly discharged in association with head movement, but their anatomical connectivity and functional role are unknown. Walton et al. (2008) reported that
inactivation of SC caused no detectable impairment of head-only movements. Thus it is difficult to comment on the likelihood that the low-frequency cells in the present report are driven by the SC cells described in Walton et al. (2007).

Of some interest is the fact that individual low-frequency cells tended to carry either head-velocity or acceleration signals but generally not both. Since both are presumably found at the level of neck-muscle motoneurons, it is possible that our low-frequency cells may represent an earlier stage of processing before these signals are combined.

The dynamic lead times of the low-frequency cells also deserve some comment. Since stimulation of nucleus reticularis gigantocellularis (NRG) evokes head movements at a mean latency of <25 ms (Quessy and Freedman 2004), our mean dynamic lead time (55 ms) may seem unexpectedly long. However, the time to peak acceleration of head movements evoked by NRG stimulation was quite similar to the distribution of lead times reported in the present study (see Table 1 of Quessy and Freedman 2004). Also, many individual low-frequency cells had dynamic lead times of <40 ms. Studies using neck-muscle EMG recording have estimated a delay of ~40 ms between the onset of muscle activity and head motion (Elsey et al. 2007). The addition of another 15–20 ms to account for neural-processing delays leads to a predicted dynamic lead time quite close to the values that we obtained in the present study. Given the much more complex musculature and dynamics of head movements, therefore, we believe that the lead times of our low-frequency cells are not implausibly long.

It is possible that some of these cells may carry signals related to dynamic motor error. However, the very low firing rates do not seem ideal for encoding dynamic variables in real time. It is hard to argue that a population of cells that typically discharges only zero to five spikes for saccades with amplitudes of <15° is involved in the dynamic, real-time control of the course of saccades. However, this possibility should probably not be excluded entirely, since the necessary temporal resolution may be possible at the population level.

It is also possible that these cells may play a role in the decomposition of gaze signals into separate eye and head commands (Freedman 2001). Since the SC clearly carries gaze-related signals, such a decomposition would need to occur somewhere downstream from this point, very likely at the level of PPRF.

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DISCLOSURES

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Author contributions: M.M.G.W. and E.G.F. conception and design of research; M.M.G.W. performed experiments; M.M.G.W. analyzed data; M.M.G.W. and E.G.F. interpreted results of experiments; M.M.G.W. prepared figures; M.M.G.W. drafted manuscript; M.M.G.W. and E.G.F. edited and revised manuscript; M.M.G.W. and E.G.F. approved final version of manuscript.

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