Physiological and Anatomical Properties of Mouse Medial Vestibular Nucleus Neurons Projecting to the Oculomotor Nucleus

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Sekirnjak, Chris and Sascha du Lac. Physiological and anatomical properties of mouse medial vestibular nucleus neurons projecting to the oculomotor nucleus. J Neurophysiol 95: 3012–3023, 2006. First published January 25, 2006; doi:10.1152/jn.00796.2005. Neurons in the medial vestibular nucleus (MVN) vary in their projection patterns, responses to head movement, and intrinsic firing properties. To establish whether neurons that participate in the vestibulo-ocular reflex (VOR) have distinct intrinsic physiological properties, oculomotor nucleus (OMN)–projecting neurons were identified in mouse brainstem slices by fluorescent retrograde labeling from the oculomotor complex and targeted for patch-clamp recordings. Such neurons were located in the magnocellular portion of the MVN contralateral to tracer injection, were mostly multipolar, and had soma diameters of around 20 μm. They fired spontaneous action potentials at rates higher than those of other MVN neurons and their spikes were of unusually short duration. OMN-projecting neurons responded to 1-s intracellular current injection with exceptionally high firing rates of >500 spikes/s. Their current–firing relationship was highly linear, with weak firing response adaptation during steady depolarization and little postinhibitory rebound firing after membrane hyperpolarization. Their firing responses were approximately in phase with sinusoidal current injection. The response dynamics of OMN-projecting neurons could be simulated with a simple integrate-and-fire model modified with the addition of small adaptation and rebound conductances. These findings indicate that the membrane properties of OMN-projecting neurons allow them to respond to head movements reliably and with high sensitivity but without substantially altering input dynamics.

INTRODUCTION

The vestibulo-ocular reflex (VOR) stabilizes images on the retina during head movements by generating compensatory eye movements. This simple behavior exhibits a number of remarkable properties. The VOR is linear and compensatory over a wide range of head motion amplitudes, velocities, and frequencies, maintaining accuracy despite responding with extremely short latencies (Huterer and Cullen 2002; Minor et al. 1999) at speeds that preclude on-line feedback control. These processing requirements make the VOR well suited to analyses of how cellular and network properties are tuned to meet behavioral demands.

Signal processing in neural circuits depends both on synaptic properties and on intrinsic membrane properties. Neurons within the central vestibular nuclei that mediate the VOR display a range of intrinsic firing properties that vary with respect to dynamic responses to current injection (Ris et al. 2001b; Sekirnjak and du Lac 2002; Straka et al. 2004; Uno et al. 2003), linearity (Beraneck et al. 2003; Ris et al. 2001b), and the ability to maintain high firing rates (Murphy and du Lac 2001). The functional significance of these variations in intrinsic properties has been difficult to assess because different classes of neurons that have been distinguished in intact animals on the basis of response properties, synaptic inputs, and axonal projections are highly intermingled in the vestibular nuclei.

The primary neural pathway that subserves the VOR constitutes a three-neuron arc in which vestibular nerve afferents synapse onto vestibular nucleus neurons, which in turn synapse onto oculomotor motoneurons. Premotor signals that drive eye movements are carried by subsets of neurons in the medial vestibular nucleus (MVN) and superior vestibular nucleus (SVN) that project to the midbrain trochlear and oculomotor nuclei (OMN) or to the abducens nucleus (Buttner-Ennever 1992; Hightsong and Holstein 2005). Vestibular nucleus neurons that project to the midbrain and subserve the VOR have been studied extensively in vivo; they have high tonic firing rates and modulate their responses linearly as a function of eye position over a wide range of firing rates (Chubb et al. 1984; Iwamoto et al. 1990; King et al. 1976; McCrea et al. 1987a; Stahl and Simpson 1995; Tomlinson and Robinson 1984).

This study examines the intrinsic properties of vestibular nucleus neurons that project to the oculomotor nucleus. Oculomotor nucleus projecting (OMP) neurons in the mouse MVN were retrogradely labeled with fluorescent tracer placed in the OMN and targeted for whole cell recordings in brainstem slices. The intrinsic firing properties of OMP neurons were then compared with a random population of unidentified MVN neurons to determine whether OMP neurons could be distinguished from others on the basis of intrinsic physiology. The results indicate that OMP neurons are well tuned to support the wide linear operating range of the VOR; OMP neurons fire over an exceptionally wide range of rates and act as linear signal processors with little intrinsic temporal filtering.

METHODS

Tracer injection

A total of 39 mice of mixed C57BL/6 and BALB/c background were used to study neurons that project to the oculomotor nucleus (OMN). For electrophysiological analyses of MVN neurons, the OMN was targeted successfully for injection in 22 of 33 mice, age 16–18 days. Recordings were obtained from brain slices obtained on the following day from 19 of these mice. Six additional adult mice

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were used for histological analyses of projections to the OMN, of which two uninjected mice served as controls for background fluorescence.

The oculomotor nucleus was stereotaxically targeted for microinjection of solid crystals of tetramethylrhodamine dextran (Molecular Probes, Eugene, OR). Crystalline tracers can be deposited at a site of interest at concentrations higher than those of liquids, are less subject to tracer leakage into the needle tract, and yield reproducible injections of larger size than is generally achieved by liquid injections (Marin et al. 2001). A microinjector equipped with a blunt 30G hypodermic needle and a moveable internal rod was loaded with several crystals of tetramethylrhodamine dextran. The needle opening was covered with melted bone wax. Animals were anesthetized with isoflurane and a hole was drilled into the exposed skull, approximately 500 μm rostral to Lambda. The microinjector needle was lowered vertically into the midbrain using a stereotaxic frame and manipulator. Thirty pulses (0.3 s at 1 Hz) of compressed nitrogen (10 ps) were delivered to the injector piston, and the resulting movement of the internal rod (100–150 μm) pushed the tracer crystals into the tissue. After a settling time of 2–3 min, the injector was retracted. The animals were killed after 12–20 h. All injection sites were later sectioned and inspected for position and size. The majority of injections (12) were located at the level of the rostral 2/3 of the oculomotor nucleus, the remainder in the caudal part.

The oculomotor nuclei of two mice were injected with red fluorescent microspheres (Lumafluor, Naples, FL), which are physiologically inert (Katz et al. 1984). The beads were injected as an undiluted solution, by use of a glass pipette with an opening diameter of 28 μm. Several 15- to 20-ms pressure pulses (15 psi) were applied to release the liquid.

Fixed tissue preparation

The animals were deeply anesthetized with sodium pentobarbital and decapitated. After removal of the brain from the skull, a portion containing the vestibular nuclei and the midbrain was dissected out and fixed overnight in 4% paraformaldehyde plus 30% sucrose. Frozen coronal sections were cut on a Microm sliding microtome at a thickness of 50–100 μm. After rinsing in 0.1 M phosphate-buffered saline, the sections were floated on glass slides, treated with an antifade gel containing 2.5% DABCO (1,4-diazabicyclo[2.2.2]octane), and coverslipped. Slices used for patch-clamp recordings were fixed and sectioned in a like manner after acquiring OMP neuron firing properties.

Imaging

Fluorescent images were recorded using a Hamamatsu CCD camera attached to an Olympus BX60 light microscope with a 4 × (NA 0.13), 10 × (NA 0.3), 40 × (NA 1.00), or 100 × (NA 1.35) objective lens. All images were collected digitally (Q.E.D. software) and transferred to Adobe Photoshop. Subdivisions of the vestibular complex followed terminology used in the Paxinos and Franklin (2001) mouse brain atlas and the study of vestibular intrinsic connections by Epema et al. (1988), in which the ventrolateral portion of the vestibular complex is designated as the magnocellular division of the MVN rather than the ventrolateral vestibular nucleus (Highstein and Holstein 2005).

Electrophysiology

The hindbrain of mice that were deeply anesthetized with Nembutal was rapidly removed from the skull and placed in ice-cold artificial cerebrospinal fluid (ACSF) aerated with 95% O2-5% CO2. A tissue block containing the brainstem with attached cerebellum was dissected away and glued to a Teflon chuck with cyanoacrylate. Coronal slices (250 μm thick) were prepared on a vibratome (Campden Instruments) in ice-cold aerated ACSF. The slices were immediately transferred to a holding chamber and incubated at 36°C for 30–60 min in aerated bath solution (ACSF).

The ACSF contained (in mM): 124 NaCl, 5 KCl, 1.3 MgSO4, 26 NaHCO3, 2.5 CaCl2, 1 NaH2PO4, and 11 dextrose. Kynurenic acid (2 mM) was added to the bath solution in all experiments to block ionotropic glutamatergic transmission. Aerated ACSF had a final pH of 7.4 and an osmolarity of 300 mOsm.

Micropipettes for whole cell recordings were fabricated from borosilicate glass (OD 1.5 mm; Garner Glass) with a Sutter Instruments P-97 puller and had resistances of 5–15 MΩ. The internal recording solution contained (in mM): 140 K-gluconate, 8 NaCl, 10 HEPES, 0.1 EGTA, 2 Mg-ATP, and 0.3 Na-GTP. The pH was adjusted to 7.2–7.5 and the osmolarity to 280–285 mOsm.

Whole cell patch recordings were performed in a submergence-type chamber with continuous perfusion of aerated ACSF. Neurons were visualized at 40–80 × with an infrared differential interference contrast (DIC) microscope (Olympus) at depths of 10–40 μm below the slice surface. The red fluorescence signal was detected using a xenon light source and an MTI-Dage CCD camera. Rate was taken to keep fluorescence illumination to a minimum and thus the xenon light was switched off as soon as positive identification of a neuron was accomplished. Recordings were made with an Axoclamp 2B amplifier (Axon Instruments) in current-clamp mode. Access resistance was checked and compensated regularly throughout each experiment. Input resistance was measured by injecting small hyperpolarizing current pulses from a membrane potential of about −80 mV. Signals were filtered at 10 kHz, digitized at 20 kHz, and recorded using a Macintosh G4 computer. Intracellular current injection of steps and sine waves was controlled by the computer. All recordings were made at a temperature of 31–33°C. A liquid junction potential of 14 mV (calculated with JPCalc) was subtracted from all membrane potentials.

Data analysis

The recorded signals were analyzed off-line using software written in Igor Pro (Wavemetrics, Lake Oswego, OR). Instantaneous firing rate was calculated as the reciprocal of the interval between successive spikes and was assigned to the time of the second spike. Action potential parameters were calculated during firing rates around 10 spikes/s (mean 11.5 ± 0.4 spikes/s) from averages of 10–20 action potentials, aligned at the peak. The width of the action potential was measured at spike threshold and the afterhyperpolarization (AHP) amplitude was calculated as the membrane potential difference between spike threshold and the absolute membrane potential minimum after the falling phase of the spike. Action potential threshold was defined as the membrane potential at which the first derivative of the membrane potential (dV/dt) exceeded 10 V/s. Action potential height was defined from threshold to peak. Neurons with action potential heights <35 mV were excluded from the analysis. The afterdepolarization (ADP) was defined as the maximum of the first derivative of the membrane potential within 1.5 ms of the peak of the action potential. Firing response gains were calculated by injecting depolarizing 1-s current steps of increasing amplitude and calculating the mean firing rate during each step. Because most MVN neurons show linear current–firing rate relationship (du Lac and Lisberger 1995a,b), the slope of a best-fit line was used to define response gain and its correlation coefficient R2 was used to define linearity. In response to large-amplitude depolarization, neurons exhibited spike inactivation (Murphy and du Lac 2001) and were not able to sustain firing for the entire 1 s stimulus. Maximum firing rate was defined as the mean firing rate evoked by the smallest current step that produced spike inactivation. Firing rate adaptation was quantified as the ratio of 100-ms averages of firing rate at the beginning and the end of each current step. Firing rate decayed exponentially after the first few interspike intervals, which varied markedly as a function of step amplitude. As with a previous study on firing rate adaptation in MVN neurons (Sekirnjak and du Lac 2002), the first 50 ms after step onset
were excluded from the calculation. Adaptation was assessed in response to current steps that produced firing rates near 40 spikes/s at the end of the step. Postinhibitory rebound firing was defined as the difference in firing rate before and immediately after a 1-s hyperpolarizing current pulse, which lowered the membrane potential on average by 30 mV. Membrane time constants were measured by fitting an exponential curve to the first 200 ms of the subthreshold responses acquired for the calculation of input resistance.

Sinusoidal functions \( f(t) = A + B \sin(2\pi ft + C) \) were fitted to the firing rate responses to sinusoidally oscillating current injections, with the parameters \( A, B, \) and \( C \) chosen to minimize the mean squared error. The frequency \( f \) was set equal to the input frequency. Phase shifts were given by the best-fit values of parameter \( C \). A stretch of at least four to five cycles of each frequency was used to obtain fits. Neurons were depolarized to fire at rates of 30–40 spikes/s before the injection of sinusoidally oscillating current. The amplitude of injected current was adjusted to modulate the firing rate by \( \pm 10–15 \) spikes/s. No inhibitory firing cutoff was evoked during any sinusoidal current injection. The frequency dependency of a first-order low-pass filter with time constant \( T \) was calculated as \( 1/(1 + (2\pi ft)^2) \), where \( f \) is the input frequency. The corresponding phase was \(-180\pi \cdot \arctan(2\pi ft)\).

To estimate cell size, images of dye-filled neurons in fixed tissue (both in adult and young animals) were analyzed. The longest axis of each cell was measured and used as soma diameter. The number of visible primary processes was counted.

Mann–Whitney U test (StatView software) was used to analyze group differences. A \( P \) value < 0.05 was considered significant. All error values given are mean \pm SE, unless otherwise indicated. All values of \( n \) stated are numbers of neurons.

**Neuronal model**

A computer model of firing rate dynamics, implemented in Igor Pro (Sekirnjak and du Lac 2002), consisted of a leaky integrate-and-fire algorithm (Knight 1972), which incorporated additional conductances to simulate postinhibitory rebound firing (PRF) and firing rate adaptation (FRA). Briefly, we used an electrical membrane model that calculates changes in membrane potential from the underlying ionic conductances and an external current. Whenever the membrane voltage exceeded spike threshold, a spike of fixed amplitude and duration was generated. For a given time step \( \Delta t \), the subthreshold membrane potential change was calculated as

\[
\Delta V = \frac{I(t)}{C} \Delta t
\]

with

\[
I(t) = \sum g_i (V_i - V(t)) + I_{leak}(t) + I_{input}(t)
\]

where \( I(t) \) is the total current, \( C \) is the input capacitance (\( C = 0.1 \) kF), \( \Delta t \) is the time step increment (\( \Delta t = 100 \) ms), \( g_i \) represents ionic conductances, \( V_i \) represents their equilibrium potentials, \( I_{leak} \) is the leak current, and \( I_{input} \) is the input current (steps or sine waves). As each spike threshold was reached, an action potential was simulated by setting the membrane voltage to 15 mV, followed by a reset to \(-60 \) mV. In each simulation, the current injection was preceded by 10 s of spontaneous or evoked firing, during which all conductances involved reached equilibrium.

To describe firing response variations in MVN neurons, two conductances were used: a rebound conductance \( g_{PRF} \) and an adaptation conductance \( g_{FRA} \). The rebound conductance activated exponentially with membrane hyperpolarizations below an activation potential \( V_{act} \)

\[
\Delta g_{PRF} = \Delta g_{PRF, max} \cdot e^{-\frac{V_{act}}{\tau_{PRF}}}
\]

with \( V_{act} = -15 \) mV, \( \lambda = 70 \) mV. The adaptation conductance \( g_{FRA} \) was increased by a fixed amount \( \Delta FRA \) after every spike. Both conductances were set to decay exponentially during the interspike interval with time constants \( \tau_{PRF} \) and \( \tau_{FRA} \), respectively. The corresponding currents were modeled using an equilibrium potential of \(-20 \) mV for rebound and \(-80 \) mV for adaptation. An intrinsic, fixed-value conductance \( g_{leak} = 0.001 \) MS with equilibrium potential of \(-35 \) mV was used to simulate spontaneous firing.

Spike threshold varied in the model as observed in experimental data traces; this was implemented by increasing threshold by a fixed amount \( \Delta \text{thresh} = 0.2 \) mV after each action potential (Liu and Wang 2001). This threshold increase was set to decay exponentially between spikes with a time constant of 100 ms. Spike threshold at the beginning of simulations was set to \(-55 \) mV.

The input current \( I_{input} \) was either a rectangular pulse of 1-s duration, or several seconds of a sinusoidally modulated waveform. Evoked firing was simulated by an additional small constant input current.

For neuronal modeling, several OMP neurons were selected whose Bode plots closely resembled those of the population average (as shown in Fig. 6D). Their firing responses to depolarizing and hyperpolarizing current pulses were used to adjust the values of \( \Delta g_{PRF, max}, \Delta FRA, \tau_{PRF}, \) and \( \tau_{FRA} \) until the model closely approximated the real neuronal responses. No further adjustments were made to describe the response to sinusoidal current injection and obtain the modeled Bode plots (shown for one neuron in Fig. 6D). The maximum rebound conductance \( \Delta g_{PRF, max} \) was \( 3 \times 10^{-5} \) MS, the maximum adaptation conductance \( \Delta g_{FRA, max} \) of \( 7 \times 10^{-6} \) MS, \( \tau_{PRF} = 800 \) ms, and \( \tau_{FRA} = 1,000 \) ms.

**RESULTS**

MVN neurons that project to the oculomotor nucleus (OMN) in mice were identified by retrograde labeling and targeted for whole cell patch recordings. We first document the location and morphology of these neurons and then describe their intrinsic firing properties.

Rhodamine dextran injections into the OMN were distributed throughout the rostrocaudal extent of the nucleus, but confined to one side. Most injections were located within a 200-\( \mu \)m radius of the center of the OMN; 11 of 23 injections also encroached on the medial longitudinal fasciculus (MLF). An example of an injection site is shown in Fig. 1A.

Injections produced extensive fluorescent labeling of neurons in the OMN and axons in the oculomotor nerve. The MLF contained numerous labeled axons that could be followed to their cell bodies of origin in the vestibular nuclei and the abducens nucleus. Figure 1B shows a retrogradely labeled neuron in a living MVN slice (asterisk). Red fluorescence (top) was used to confirm neuronal identity as OMN projecting (OMP) and the corresponding DIC image (bottom) was used to subsequently target such neurons for whole cell recordings.

**Distribution and morphology of OMP neurons in the vestibular nuclei**

All successfully injected mice yielded a stereotypical pattern of retrogradely labeled cell bodies in the brain stem. Figure 1C shows tracer transport and neuronal labeling in the MVN and surrounding brain stem nuclei. Two dense clusters of labeled neurons were apparent: one in the ipsilateral superior vestibular nucleus (SVN) and one in the contralateral MVN. A small number of neurons on the lateral margin of the rostral MVN ipsilateral to the injection site were also labeled. Numerous neurons in the contralateral abducens nucleus and a small
number of neurons in the prepositus nucleus and contralateral SVN were retrogradely labeled from OMN injections. No differences were observed in the pattern of vestibular nucleus neurons that were retrogradely labeled from injections that were restricted to the OMN versus those in which dye spread to the MLF. Within the MVN, retrogradely labeled neurons clustered in the magnocellular division (Fig. 1C). The OMP neurons targeted for electrophysiological recordings in this study were distributed from 6.0 to 6.5 mm caudal to bregma, 0.7 to 1.3 mm lateral to the midline, and 4.1 to 4.4 mm ventral to the horizontal plane passing through bregma and lambda, as defined in the mouse brain atlas (Paxinos and Franklin 2001).

Figure 2A shows the somatodendritic morphology of three representative MVN neurons that were retrogradely labeled from the contralateral OMN. OMP neurons had multipolar dendritic morphologies and large cell bodies relative to neighboring unlabeled neurons. A quantitative morphological analysis of 249 retrogradely labeled neurons from five animals is shown in Fig. 2, B and C. The mean soma diameter was 22.1 ± 0.3 μm and the mean number of primary processes emanating from the cell body was 3.7 ± 0.1. As shown in the histogram in Fig. 2B, very few neurons with soma diameters <15 μm were retrogradely labeled. A small number of neurons had a bipolar dendritic morphology, whereas the majority of neurons had three or more processes (Fig. 2C).

**Intrinsic firing properties of OMP neurons in the MVN**

To examine the intrinsic firing properties of OMP neurons, we made whole cell patch recordings from 38 retrogradely
labeled neurons in slices obtained from 19 mice, age 17–19 days. The neurons were identified by brief epifluorescent illumination and targeted for recordings under infrared/DIC optics. A random assortment of 112 unidentified neurons recorded throughout the rostral two thirds of the MVN served as a comparison population.

The firing properties of neurons retrogradely labeled with rhodamine dextran were indistinguishable from those of neurons retrogradely labeled with inert fluorescent microspheres, indicating that the nature of the fluorophore did not affect intrinsic physiology. Neurons labeled from injections that partially included the MLF and those from injections that spared the MLF showed no significant difference in any of the firing properties analyzed here; thus the results presented below include data pooled from all injections.

**Spontaneous firing and action potential shape in OMP neurons**

Most OMP neurons (29/38) fired spontaneous action potentials even when excitatory synaptic transmission was blocked with kynurenic acid. Figure 3A shows spontaneous action potentials in an OMP neuron firing at 22 spikes/s. Spontaneous firing rates of OMP neurons tended to be higher than those of unidentified neurons (Table 1). To facilitate the comparison, spontaneous firing rates are shown in histogram form in Fig. 3B for 29 spontaneously active OMP and 85 unidentified neurons. The majority of unidentified neurons (light gray) fired at rates <10 spikes/s, whereas most OMP neurons (dark gray) fired between 10 and 20 spikes/s. For 21 of the spontaneously firing OMP neurons, extracellular firing rate was measured in the cell-attached patch mode before establishing the whole cell recording configuration. The mean extracellular rate for these neurons (19.1 ± 2.7 spikes/s) was similar to the intracellular firing rate (20.3 ± 2.4 spikes/s), indicating that the high spontaneous firing rates of OMP neurons was not an artifact of intracellular dialysis from the recording pipette.

Figure 4A shows an expanded view of the action potential of an OMP neuron. The rapid repolarizing phase was followed by an AHP that reached a minimum within 0.7 ms of the peak of the action potential. A brief rise in the membrane potential, termed afterdepolarization (ADP), separated the initial AHP and a slower, subsequent AHP. The trajectory of the membrane potential after the action potential varied with firing rate, as described previously (du Lac and Lisberger 1995b). To standardize the comparison across neurons, action potentials were obtained when neurons were firing at 10 spikes/s, as described in METHODS.

Action potentials were of remarkably brief duration in OMP neurons. Figure 4B plots action potential width versus the latency from the action potential to the peak of the AHP in 36 OMP neurons and 100 unidentified neurons. Action potential widths ranged from 0.52 to 1.0 ms in OMP neurons and from 0.48 to 1.7 ms in unidentified neurons and were significantly narrower in OMP than in unidentified neurons (P < 0.001, Table 1). Both the rising and the falling phases of the action potential were shorter in OMP than those in unidentified neurons (P < 0.001, Table 1). In OMP neurons, action potential fall times were 81 ± 3% shorter than action potential rise times, whereas in unidentified neurons action potential rise and fall times were similar (99 ± 2%; P < 0.001, Table 1). Action

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**FIG. 3.** Spontaneous firing in OMP neurons. A: spontaneous firing in an OMP neuron. Membrane potential is plotted as a function of time; dashed line indicates −50 mV. Mean firing rate was 22 spikes/s. B: histogram of spontaneous firing in OMP neurons and a population of age-matched unidentified MVN neurons. Neurons without spontaneous activity were excluded from this plot.
potential threshold, defined as the membrane potential at which the rate of depolarization reached 10 mV/ms, was significantly lower in OMP neurons (−51.8 ± 0.6 mV) than in unidentified neurons (−48.8 ± 0.4 mV; *P* < 0.001). These data suggest substantial differences in the ionic conductances that govern action potential generation in OMP versus other types of MVN neurons.

The latency from the action potential to the peak of the AHP varied widely across MVN neurons but was restricted to two ranges in OMP neurons: either <1.5 ms (9/36 neurons) or >5 ms (27/36 neurons) after the peak of the action potential (Fig. 4B). No OMP neurons displayed AHP peaks between 1.5 and 5 ms, in contrast with 20/100 unidentified neurons (*P* < 0.001, Fisher exact test). Examples of action potentials from pairs of OMP and unidentified neurons with early and late peak AHP times are shown in Fig. 4C. A small ADP is evident in both of the OMP neurons but in neither of the unidentified neurons. In the sample analyzed for this study, 27/36 (75%) of OMP neurons exhibited an ADP, in contrast with 25/100 unidentified neurons (*P* = 0.001, Fisher exact test, and Table 1). All OMP neurons with AHP peaks <1.5 ms exhibited an ADP.

The magnitude and time course of the AHP varied considerably across neurons. OMP and unidentified neurons in which the AHP peaked late (>5 ms after the action potential) exhibited biphasic AHPs such as those shown on the right of each spike pair in Fig. 4C. In contrast, all neurons with intermediate times to peak AHP (1.5–5 ms after the action potential) exhibited monophasic AHPs, as exemplified by the unidentified neuron on the left in Fig. 4C. Neurons with early AHP peaks (<1.5 ms after the action potential) exhibited small AHPs that typically resembled that shown for the leftmost OMP neuron in Fig. 4C. Unidentified neurons tended to have larger AHPs than OMP neurons, but the population difference was not significant (Table 1). Unidentified neurons with intermediate AHP times, however, had AHPs that were significantly larger than those in OMP neurons (25.6 ± 1.0 vs. 19.3 ± 0.5, *P* < 0.0001) as well as in unidentified neurons with early or late AHP times (19.9 ± 0.5, *P* < 0.0001).

**TABLE 1. Intrinsic membrane and firing parameters for OMP neurons and unidentified neurons**

<table>
<thead>
<tr>
<th></th>
<th>Unidentified Neurons</th>
<th>OMP Neurons</th>
<th><em>P</em>-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input resistance (MΩ)</td>
<td>281 ± 23</td>
<td>143 ± 13</td>
<td>0.0002</td>
</tr>
<tr>
<td>Spontaneous rate (spikes/s)</td>
<td>11 ± 1</td>
<td>22 ± 3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Spike height (mV)</td>
<td>53.3 ± 0.8</td>
<td>52.5 ± 1.2</td>
<td>0.81</td>
</tr>
<tr>
<td>Spike width (ms)</td>
<td>0.99 ± 0.02</td>
<td>0.72 ± 0.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Spike rise time (ms)</td>
<td>0.50 ± 0.01</td>
<td>0.40 ± 0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Spike fall time (ms)</td>
<td>0.49 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ADP (mV/ms)</td>
<td>−2.3 ± 0.04</td>
<td>1.5 ± 0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AHP (mV)</td>
<td>21 ± 0.5</td>
<td>19.2 ± 0.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Firing response gain (spikes/s·nA⁻¹)</td>
<td>189 ± 9</td>
<td>196 ± 9</td>
<td>0.1296</td>
</tr>
<tr>
<td>IF curve linearity (R²)</td>
<td>0.988 ± 0.001</td>
<td>0.994 ± 0.01</td>
<td>0.006</td>
</tr>
<tr>
<td>Maximum rate (spikes/s)</td>
<td>180 ± 10</td>
<td>327 ± 15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gain/Input resistance</td>
<td>1.1 ± 0.09</td>
<td>1.65 ± 0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FRA ratio</td>
<td>0.88 ± 0.01</td>
<td>0.89 ± 0.01</td>
<td>0.92</td>
</tr>
<tr>
<td>PRF (spikes/s)</td>
<td>16 ± 2</td>
<td>12 ± 1</td>
<td>0.89</td>
</tr>
<tr>
<td>Phase at 0.25 Hz (deg)</td>
<td>8.1 ± 1.0</td>
<td>4.3 ± 0.3</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Averages and standard errors are provided for each of the properties measured in this study. AHP, afterhyperpolarization; ADP, afterdepolarization; IF, current-firing; FRA, firing rate adaptation; PRF, post-inhibitory rebound firing; OMP, oculomotor nucleus projecting neurons.

**FIG. 4. Action potentials in OMP and unidentified neurons.** A: action potential of an OMP neuron; membrane potential of 44 spikes were averaged and aligned at action potential peak. Dashed line indicates −50 mV. B: action potential width in OMP and unidentified neurons is plotted vs. the time to the peak of the AHP in each neuron. Filled black circles: OMP neurons. Open triangles: unidentified neurons. C: 2 examples each of action potentials in OMP neurons and unidentified neurons.
Firing and membrane responses to current injection

The intrinsic firing responses of OMP neurons to membrane excitation were assessed by monitoring changes in firing rate evoked with steps of depolarizing current injected through the recording electrode. Figure 5A shows firing responses of an individual OMP neuron to a family of current steps that ranged between 0 and 2.4 nA. Firing rate increased at the onset of each depolarizing current step, declined over the course of 1 s of depolarization, and returned to the baseline level after the offset of depolarization. The decline in firing rate during sustained depolarization was quantified as the adaptation ratio (see METHODS). The adaptation ratio in OMP neurons averaged 0.89 ± 0.01, which was not significantly different from that in unidentified MVN neurons (Table 1).

OMP neurons fired linearly as a function of depolarization amplitude. Figure 5B plots the mean firing rate during depolarization versus input current for the neuron shown in Fig. 3A. The correlation coefficient $R^2$ was 0.99 in this neuron and ranged between 0.977 and 0.999 across all OMP neurons (Table 1). The gain of the firing response was defined as the slope of the best linear fit to the firing rate–current relationship. Firing response gains in OMP neurons ranged from 101 to 335 (spikes/s)/nA and were not significantly different from gains in unidentified neurons (Table 1).

OMP neurons were capable of sustaining remarkably high firing rates. In the example shown in Fig. 5B, the mean firing rate in response to depolarization with 2.4 nA of current was 450 spikes/s. Larger amplitudes of depolarization resulted in spike inactivation, which likely reflects cumulative sodium channel inactivation (Erisir et al. 1999; Murphy and du Lac 2001). Figure 5C plots the maximum firing rates that could be sustained in OMP and unidentified neurons versus each neuron’s input resistance. On average, OMP neurons could fire almost twice as fast as the average unidentified MVN neuron (see Table 1). The rapid rise and repolarization of the action potential in OMP neurons may contribute to these high firing rates because neurons with the greatest maximum firing rates tended to have the narrowest action potentials (correlation $R^2 = 0.54$).

Input resistance varied widely in unidentified neurons but tended to be low in OMP neurons (Fig. 5C); mean input resistances were 281 ± 23 in unidentified neurons versus 143 ± 13 in OMP neurons (Table 1, $P = 0.002$). Unidentified neurons with high maximum firing rates also tended to have low input resistances but, unlike OMP neurons, many unidentified neurons with low input resistances had low maximum firing rates (Fig. 5C). OMP neurons were more excitable in the firing domain than would be expected from their low input resistances. As shown in the histograms in Fig. 5D, the ratio of excitability above and below spike threshold was significantly greater in OMP neurons than in unidentified neurons ($P < 0.0001$, Table 1).

Intrinsic firing dynamics in OMP neurons

The dynamics of intrinsic firing responses vary considerably across MVN neurons (Beraneck et al. 2003; Ris et al. 2001b; Sekirnjak and du Lac 2002). In a previous study of unidentified mouse MVN neurons, the firing response adaptation ratio correlated strongly both with postinhibitory rebound firing and with the dynamics of firing responses to sinusoidally modulated current (Sekirnjak and du Lac 2002). OMP neurons recorded in the present study exhibited relatively little postinhibitory rebound firing, as evidenced by the example shown in Fig. 6A. Hyperpolarizing current silenced the neuron and resulted in a depolarizing “sag” of the membrane potential (arrow in Fig. 6A) that is a hallmark of the hyperpolarization-activated current $I_{h}$. After the offset of hyperpolarization, the neuron’s firing rate returned to the spontaneous level without rebounding transiently to a higher level. Peak postinhibitory rebound firing was quantified as the difference between the
The gain of firing rate modulation (peak-to-peak firing rate versus input current) was analyzed from sinusoidal responses to input frequencies between 0.125 and 4 Hz. The firing rate immediately after hyperpolarization and that before hyperpolarization, and is plotted in Fig. 6B as a function of the adaptation ratio during depolarization for the population of OMP neurons and the unidentified MVN neurons recorded in a previous study (Sekirnjak and du Lac 2002). OMP neurons clustered with MVN neurons that displayed little or no rebound firing nor adaptation.

Because head motion signals in the behaving animal give rise to alternating depolarizing and hyperpolarizing drive to MVN neurons, we analyzed intrinsic firing responses to sinusoidally varying current. Figure 6C shows modulation in firing rate in an individual OMP neuron in response to sinusoidally modulated current around a baseline firing rate of 36 spikes/s. The gain of firing rate modulation (peak-to-peak firing rate divided by peak-to-peak current amplitude) and the phase of firing relative to the input signal were analyzed from sinusoidal responses to input frequencies between 0.125 and 4 Hz. The resulting Bode plots for 22 OMP neurons are presented in Fig. 6D. Gain increased modestly with input frequency, whereas the response phase tended to decrease with frequency. Phase values measured at 0.25 Hz were significantly lower in OMP neurons than in unidentified MVN neurons (Table 1).

Firing response dynamics in OMP neurons were not predicted by subthreshold membrane dynamics. The dashed lines in Fig. 6D indicate the gain and phase of an ideal low-pass filter, predicted from the average membrane time constant in OMP neurons (10.7 ± 0.8 ms, n = 36). Predicted subthreshold gain was relatively flat across frequency and decreased at the highest frequencies; in contrast, the actual suprathreshold firing response gain increased with stimulus frequency. Predicted subthreshold phase decreased with stimulus frequency in a fashion similar to that of actual firing response phase. The differences in subthreshold and suprathreshold dynamics observed in OMP neurons indicate that membrane conductances active during firing boost the intrinsic neuronal response to high-frequency stimuli.

In a previous study on firing dynamics in unidentified MVN neurons, modifications to a simple integrate-and-fire model were able to reproduce variations across neurons in phase responses to sinusoidal stimuli (Sekirnjak and du Lac 2002). The addition of spike- and hyperpolarization-triggered conductances boost the gain and advance the phase of conventional integrate-and-fire models, which produce firing dynamics that resemble low-pass filters (du Lac and Lisberger 1995a). The firing responses of OMP neurons were simulated with an integrate-and-fire model that incorporated small adaptation and rebound conductances. As shown in Fig. 6D (dotted lines), the model closely approximated the firing response dynamics of real OMP neurons.

**Discussion**

This study investigated the anatomical and physiological properties of mouse MVN neurons projecting to the contralateral OMN. Such projection neurons were found to be concentrated in the magnocellular portion of the MVN and largely constituted medium-sized multipolar neurons. They were capable of firing at unusually high rates and transformed current injections linearly into firing responses with little temporal filtering. These findings imply that the membrane properties of MVN neurons projecting to the oculomotor nucleus are well suited for rapid transmission of vestibular signals over a wide range of amplitudes and frequencies.

**Medial vestibular nucleus projections to the oculomotor nucleus**

Similar to findings from tracer studies in rabbit (Labandeira-Garcia et al. 1991; Wentzel et al. 1995), cat (Gacek 1977;
Graybiel and Hartwig 1974), rat (Saxon and Beitz 2000), chick (Labandeira-Garcia et al. 1989), and monkey (Carpenter and Carleton 1983; McCrea et al. 1987a), OMP neurons in the mouse constitute spatially localized bands of neurons centered primarily in the magnocellular division of the MVN contralateral and the SVN ipsilateral to the injection site. The small number of retrogradely labeled neurons found in the lateral portion of the MVN ipsilateral to the injection site may correspond to neurons projecting through the ascending tract of Deiters (ATD) that have been described in cat (Baker and Highstein 1978; Carleton and Carpenter 1983; Furuya and Markham 1981; Gacek 1977; Nguyen et al. 1999; Reisine et al. 1981), rat (Nagata 1986), and monkey (Carpenter and Carleton 1983; McCrea et al. 1987b). A sparse projection from the nucleus prepositus to the OMN has been reported in rabbits (Labandeira-Garcia et al. 1990) and cats (McCrea and Baker 1985), consistent with the few labeled neurons found in this study. The projection from the MVN to the OMN arises from medium and large neurons in the rostral half of the nucleus in cat (Gacek 1977) and monkey (McCrea et al. 1987a,b), in agreement with our findings in the mouse. The origination of OMP neurons in the magnocellular division of the MVN is consistent with previously established patterns of intrinsic and extrinsic vestibular nucleus neuronal connectivity, in which commissural and intrinsic projections originate predominantly from the caudal and parvocellular divisions of the MVN, whereas the majority of ocular motor and spinal projections originate in the magnocellular division or ventrolateral vestibular nucleus (Buttner-Ennever 1992; Epema et al. 1988).

We cannot exclude the possibility that some neurons recorded in this study were vestibulothalamic neurons, whose axons also travel in the MLF (Lang et al. 1979; Maciewicz et al. 1982; Nagata 1986) and may have picked up the fluorescent tracer in a subset of injections. However, several studies have demonstrated that canal-activated secondary vestibular nucleus neurons projecting to the OMN often continue to more rostral regions (Furuya and Markham 1981) and give off collaterals to the thalamus (Matsuo et al. 1994, 1995). It is thus conceivable that some OMP neurons in this study also have higher ascending projections. Consistent with this idea, we found no different properties in neurons from injections that were restricted to the OMN and those that included the MLF.

Two primary pathways are subserved by neurons projecting from the medial or ventrolateral vestibular nucleus to the OMN: a pathway from the posterior canal to the contralateral inferior rectus muscle (Matsuo et al. 1994; McCrea et al. 1987a) and one from the horizontal canal to the ipsilateral medial rectus muscle (Highstein 1973; Ito et al. 1976; Kawaguchi 1985; Tarlov 1970). Because all neurons in this study were recorded from the contralateral MVN, we presume that they belong to the posterior canal pathway that relates vertical head motion to the motor neurons serving vertical eye movements. The majority of midbrain-projecting neurons in the vestibular nuclei receive monosynaptic input from the ipsilateral vestibular nerve (Fukushima et al. 1990). OMP neurons likely correspond to vertical position–vestibular-pause (PVP) cells identified in vivo (Chubb et al. 1984; Iwamoto et al. 1990; King et al. 1976; McCrea et al. 1987a; Pola and Robinson 1978), which receive direct vestibular nerve input (Fukushima et al. 1990), project to the OMN (McCrea et al. 1987a), and have a multipolar dendritic morphology (McCrea et al. 1987a).

Midbrain-projecting neurons in the MVN that are inhibited by the cerebellar flocculus display sensitivity to horizontal head and/or eye movements (Kawaguchi 1985; Stahl and Simpson 1995), suggesting the possibility that OMP neurons may be involved in the control of horizontal as well as vertical eye movements.

Classification of MVN neurons in vitro

MVN neurons constitute a heterogeneous population that differs with respect to their axonal projections, synaptic inputs, and physiological response properties in vivo. A major challenge for assessing the functional significance of cellular properties of vestibular nucleus neurons is to relate the cell types identified in vivo to neurons recorded in vitro. Initial attempts to classify cell types in brain slices focused on variations in spike shape. MVN neurons were divided into two main types, primarily based on the action potential repolarization and the afterhyperpolarization (AHP): type A neurons repolarize slowly and exhibit a monophasic AHP, whereas type B neurons repolarize rapidly and exhibit a biphasic AHP (Johnston et al. 1994; Serafin et al. 1991). Type B neurons were subdivided into multiple subtypes on the basis of the presence or absence of plateau potentials and low-threshold spiking (Him and Dutia 2001; Serafin et al. 1991). Subsequent analyses of intrinsic firing dynamics indicated that MVN neurons are heterogeneous in their firing responses to sinusoidal current injection (Ris et al. 2001b; Sekirnjak and du Lac 2002), firing response adaptation (Ris et al. 2001b; Sekirnjak and du Lac 2002), and postinhibitory rebound firing (Sekirnjak and du Lac 2002). Although neurons categorized as type B tend to have more high-pass dynamics than those categorized as type A (Ris et al. 2001b), intrinsic response dynamics appear to vary continuously (Sekirnjak and du Lac 2002), necessitating additional strategies for unambiguously classifying MVN neurons into functionally distinct types.

A recent advance in classifying MVN neurons according to function, rather than spike shape, was made by combining single-cell PCR analysis of transmitter phenotype with whole cell recording in the dorsal MVN (Takazawa et al. 2004). Interestingly, glutamatergic neurons formed a relatively homogeneous population that exhibited continuous firing during depolarizing current injection, a biphasic AHP, an ADP, and a prominent membrane potential sag in response to subthreshold current injection. In contrast, GABAergic neurons were heterogeneous and included both neurons with a biphasic AHPs as well as neurons with monophasic AHPs that exhibited delayed firing responses to depolarization.

The present study used retrograde labeling from the oculomotor nucleus to target physiological recordings to a specific class of MVN neurons. The intrinsic properties of this population of retrogradely labeled neurons were largely homogeneous relative to unidentified MVN neurons.

The action potential shape and membrane potential response during hyperpolarization of OMP neurons match those of the glutamatergic neurons identified in the dorsal MVN by Takazawa et al. (2004), consistent with the excitatory nature of vestibular nucleus projections to the OMN (Baker and Highstein 1978; Matsuo et al. 1994; Wentzel et al. 1995). OMP neurons repolarized rapidly and had biphasic AHPs; as such, they would be classified as type B on the basis of spike...
Candidate ionic currents in OMP neurons

Differences in the intrinsic physiological properties of OMP and other MVN neurons are likely to reflect heterogeneity in the ion channels expressed in each cell type. Previous pharmacological analyses of unidentified MVN neurons provide insights into candidate ionic currents in OMP neurons. The late component of the biphasic AHP is consistent with the presence of a prominent SK-type calcium-dependent potassium current (Johnston et al. 1994; Smith et al. 2002). In MVN neurons, the membrane sag during subthreshold hyperpolarization, which is pronounced in OMP neurons, is mediated by the hyperpolarization-activated cationic current $I_{h}$ (Sekirnjak and du Lac 2002). The ability of OMP neurons to sustain high firing rates in response to depolarizing current, together with rapid action potential repolarization, indicates the presence of potassium and sodium currents that are specialized to support rapid firing. Kv3 channels, which are expressed in fast-firing neurons (Gan and Kaczmarek 1998; Rudy and McBain 2001) are abundant in MVN neurons (Weiser et al. 1995) and may be particularly strongly expressed in OMP neurons. The resurgent sodium current, which supports rapid repolarization and fast firing in a number of types of neurons (Afsahari et al. 2004; Raman and Bean 1997), appears to be expressed in a subset of unidentified MVN nucleus neurons that exhibit rapid action potential repolarization (AG Gittis and S du Lac, unpublished results). Ultimately, analyses of the strengths and distributions of different ionic conductances in distinct cell types such as OMP neurons will be useful not only for neuronal classification but moreover for understanding the mechanisms with which experience regulates the intrinsic excitability of identified vestibular nucleus neurons (Darlington et al. 2002; Him and Dutia 2001; Nelson et al. 2003; Park and Jeong 2000; Ris et al. 2001a,c).

Functional implications

The VOR is remarkably linear over a wide operating range of head movement amplitudes and frequencies. The OMP neurons recorded in this study had intrinsic firing properties that appear to be optimized for linear spike generation over an exceptionally wide operating range. OMP neurons fired spontaneously at high rates in vitro and responded to input currents with linear changes in firing rate over a wide range of inputs and firing rates. Responses to step and sinusoidally injected current indicated that OMP neurons transform their inputs into firing rate modulations with relatively little temporal filtering. These intrinsic properties make OMP neurons well suited to respond faithfully to head and eye motion over a wide range of amplitudes and frequencies. In vivo, vestibular nucleus neurons that project to the oculomotor nucleus or that have axons that course rostrally in the MLF fire tonically at high rates that modulate sinusoidally with sinusoidal head rotation (Chubb et al. 1984; Iwamoto et al. 1990; King et al. 1976) and linearly as a function of eye position (Chubb et al. 1984; Iwamoto et al. 1990; King et al. 1976) and linearly as a function of eye position (Chubb et al. 1984; Iwamoto et al. 1990; King et al. 1976; McCrea et al. 1987a; Stahl and Simpson 1995; Tomlinson and Robinson 1984). The intrinsic linearity of the OMP spike generator implies that synapses conveying eye position, eye velocity, and head velocity signals onto these neurons also operate linearly over a wide range of stimulus conditions.

Our findings have implications for computational models of the VOR. Ionic conductances that are active during firing are critical for determining intrinsic response dynamics, which cannot be predicted from the membrane time constant obtained below spike threshold (du Lac and Lisberger 1995a; Ris et al. 2001b; Sekirnjak and du Lac 2002). Simple integrate-and-fire models are thus inadequate descriptors of the firing responses of OMP neurons. One alternative approach is conductance-based Hodgkin–Huxley (HH) type models, which have been used to describe intrinsic dynamics of MVN neurons (Av-Ron and Vidal 1999; Quadroni and Knapfel 1994) but require detailed and typically unavailable information about the voltage, time, and calcium dependency of each conductance. A simplified HH model incorporating two voltage-dependent potassium conductances could simulate variations in intrinsic dynamics across MVN neurons (Ris et al. 2001b). We have found that the firing dynamics of MVN neurons can be well described by incorporating ionic conductances into an inte-
grate-and-fire model (Sekirnjak and du Lac 2002). In the case of OMP neurons, response dynamics could be reproduced by the addition of a spike-triggered outward current and a small hyperpolarization-triggered inward current. This modified description of the integrate-and-fire model can be readily incorporated into network models of oculomotor function that capture intrinsic physiological properties while remaining computationally tractable.

In summary, by targeting recordings to neurons retrogradely labeled from the OMN, this study has revealed the intrinsic properties of a functionally distinct population of MVN neurons. OMP neurons can be identified by their location in the magnocellular portion of the MVN, their predominantly multipolar dendritic morphology, and by their combination of high maximal firing rates during steady depolarization and minimal rebound firing after hyperpolarization. Their highly linear firing responses over a wide range of inputs indicate that OMP neurons express ionic conductances that are well tuned for the rapid and accurate control of eye movements.

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