Anatomy and Discharge Properties of Pre-Motor Neurons in the Goldfish Medulla That Have Eye-Position Signals During Fixations

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Aksay, E., R. Baker, H. S. Seung, and D. W. Tank. Anatomy and discharge properties of pre-motor neurons in the goldfish medulla that have eye-position signals during fixations. J Neurophysiol 84: 1035–1049, 2000. Previous work in goldfish has suggested that the oculomotor velocity-to-position neural integrator for horizontal eye movements may be confined bilaterally to a distinct group of medullary neurons that show an eye-position signal. To establish this localization, the anatomy and discharge properties of these position neurons were characterized with single-cell Neurobiotin labeling and extracellular recording in awake goldfish while monitoring eye movements with the scleral search-coil method. All labeled somata (n = 9) were identified within a region of a medially located column of the inferior reticular formation that was ~350 μm in length, ~250 μm in depth, and ~125 μm in width. The dendrites of position neurons arborized over a wide extent of the ventral half of the medulla with especially heavy ramification in the initial 500 μm rostral of cell somata (n = 9). The axons either followed a well-defined ventral pathway toward the ipsilateral abducens (n = 4) or crossed the midline (n = 2) and projected toward the contralateral group of position neurons and the contralateral abducens. A mapping of the somatic region using extracellular single unit recording revealed that position neurons (n > 120) were the dominant eye-movement-related cell type in this area. Position neurons did not discharge below a threshold value of horizontal fixation position of the ipsilateral eye. Above this threshold, firing rates increased linearly with increasing temporal position [mean position sensitivity = 2.8 (spikes/s)/°, (n = 44)]. For a given fixation position, average rates of firing were higher after a temporal saccade than a nasal one (n = 19/19); the magnitude of this hysteresis increased with increasing position sensitivity. Transitions in firing rate accompanying temporal saccades were overshooting (n = 43/44), beginning, on average, 17.2 ms before saccade onset (n = 17). Peak firing rate change accompanying temporal saccades was correlated with eye velocity (n = 36/41). The anatomical findings demonstrate that goldfish medullary position neurons have somata that are isolated from other parts of the oculomotor system, have dendritic fields overlapping with axonal terminations of neurons with velocity signals, and have axons that are capable of relaying commands to the abducens. The physiological findings demonstrate that the signals carried by position neurons could be used by motoneurons to set the fixation position of the eye. These results are consistent with a role for position neurons as elements of the velocity-to-position neural integrator for horizontal eye movements.

INTRODUCTION

The velocity-to-position neural integrator (VPNI) is a hypothesized part of the oculomotor system that implements time integration in the mathematical sense, transforming velocity-encoding neural inputs into position-encoding outputs (Robinson 1975, 1989; Skavenski and Robinson 1973). During saccades, VPNI neurons should integrate a pulse-like velocity signal from saccadic burst neurons into a sustained change in their own firing rate, thus providing the position signal needed to hold the eye at a new fixation point. The physiological processes by which transient inputs could be transformed into sustained changes in neural activity may include precisely tuned synaptic feedback in recurrent networks (Cannon and Robinson 1985; Cannon et al. 1983; Seung 1996), and cellular properties such as short-term synaptic plasticity (Shen 1989). To assess the potential role played by these mechanisms, the neuronal elements of the VPNI must first be identified.

Two medullary nuclei in mammals, the nucleus prepositus hypoglossi (NPH) and medial vestibular nucleus (MVN), have been implicated as sites of the VPNI for horizontal eye movements. In support of this hypothesis, the NPH and MVN contain appropriate input and output pathways to mediate integration of vestibular and saccadic commands (Fukushima et al. 1992; McCrea 1988; Moschovakis 1997). A subpopulation of neurons in the NPH and MVN show sustained neural activity during fixations (Baker et al. 1975; Lisberger et al. 1994; Lopez-Barneo et al. 1982; McConville et al. 1994; McFarland and Fuchs 1992). Below a threshold eye position, these neurons are silent. Above this threshold, firing rate is a linear function of horizontal eye position. Lesion and pharmacological inactivation of regions including the MVN/NPH induced profound centripetal drift during horizontal fixations and impairment of the vestibuloocular reflex (VOR) and optokinetic reflex (OKR) that is consistent with loss of integrator function (Cannon and Robinson 1987; Cheron et al. 1986; Moreno-Lopez et al. 1996). Recently permanent lesions restricted to the rostral NPH in the primate led to deficits in horizontal fixations but largely spared horizontal sinusoidal VOR, suggesting that integration of vestibular commands is primarily performed elsewhere (Kaneko 1997, 1999).

While mammalian systems have played a central role in efforts to localize the VPNI, the goldfish oculomotor system may prove advantageous in elucidating the mechanisms by which the neural integrator operates. Both intracellular recording and optical imaging can be performed in the awake goldfish.
to provide information about synaptic currents and intrinsic electrophysiological properties in the native neuromodulatory environment (Aksay et al. 1998; Graf et al. 1997; Suwa et al. 1996; Svoboda et al. 1997). Furthermore since goldfish eye movements include a spontaneous scanning pattern of horizontal saccades and fixations, the performance of the neural integrator can be assessed without any behavioral training. In this system, two hindbrain regions, termed area I and area II, have been implicated in oculomotor signal generation and storage (Pastor et al. 1994). Neurons in area II have firing rates that modulate in phase with eye velocity during VOR and OKR and do not change with fixation position, rendering it unlikely that this region is part of the VPNI. Rather, lidocaine inactivation studies suggest that it is a site for generation and storage of eye-velocity signals. In contrast, within area I, the more caudal of the two regions, neurons show eye-position signals. In addition, lidocaine inactivation of regions including area I induced profound centripetal drift during horizontal fixations and significant impairment of low-frequency (<0.25 Hz) VOR (Pastor et al. 1994). Hence it is likely that area I composes at least part of the goldfish VPNI for horizontal eye movements. The work presented here is directed toward characterizing the anatomical and firing rate properties of neurons in area I that show an eye-position signal. These neurons will be referred to as “position” neurons.

If area I is indeed an element of the VPNI, then it should have the appropriate input and output connectivity to mediate velocity-to-position integration. Neurons of the VPNI should have a soma/dendritic field that overlaps with the axonal termination fields of eye-velocity-signaling neurons. They should also have axonal projections terminating on the soma/dendritic fields of the abducens motoneurons and interneurons. Therefore electrophysiologically identified position neurons in awake goldfish were labeled with Neurobiotin to visualize their somatic location, dendritic arborization, and axonal projection patterns.

To interpret the role of position neurons in behavioral deficits induced by lesion or pharmacological inactivation of area I (Pastor et al. 1994), it is necessary to determine if other eye-movement-related neurons are located within the same region. Therefore a careful mapping of cell types within the medulla was performed using extracellular single-unit recording. The spatial extent of neurons with eye-position signals was determined, and the spatial segregation of these neurons from other oculomotor neuronal types was established.

During fixations, the output of the horizontal VPNI is expected to provide a position signal to the abducens; hence, VPNI neuron firing rates should correlate strongly with horizontal eye position. Therefore the relationship between firing rate and eye position during fixations was quantified for position neurons in area I. Also, hysteresis in the rate-position relationship, latency of change in neuronal activity to the onset of saccades, and sensitivity to saccade velocity were quantified to investigate the contribution of position neuron signals to the activity of abducens neurons during saccades and fixations. All properties were studied in both light and dark conditions to assess the role of visual feedback on position neuron activity.

Portions of this work have been presented elsewhere in abstract form (Aksay et al. 1997).
Single-unit electrophysiology and spike detection

RECORDING METHODS. Single-unit recordings were made with glass microelectrodes filled with extracellular recording solution [2 M NaCl, 10 mM Fast Green (F-99, Fisher Scientific), pH = 7.4] and beveled to a resistance between 2 and 5 MΩ. This range in tip size was optimal for obtaining well-isolated, high signal-to-noise ratio (>3:1) recordings. Preamplified signals were band-pass filtered (300 Hz to 10 kHz) prior to being digitized at 20.00 kHz (16.67 kHz for dual recordings). Acquisitions were made either with a 12 bit A-D MacAdios board (GW Instruments, Somerville, MA) and custom software or a Digidata 1200B data-acquisition system and Clampex 7.0 software (Axon Instruments, Foster City, CA). In both cases, eye movements and electrophysiological recordings were digitized on the same system to ensure synchronization of data over long recording periods.

SPIKE DETECTION. Digitized voltage traces were processed off-line by custom spike-detection software written in Matlab. Events exceeding a negative voltage threshold (typically −50 to −150 μV) were detected to initially select action potential waveforms. Two parameters of detected spike shape, the peak-to-peak amplitude and the time separation between peaks, were plotted against each other, and a bounded region defining a single cluster of points was specified. The times of all spikes within the boundary were used in subsequent data analyses. Comparison of detected spike times with the original voltage trace indicated that the number of inappropriately omitted or included spikes was <1% of the total.

IDENTIFICATION OF AREA 1. Electrodes were advanced into the hindbrain along vertical tracks aligned with the dorsal-ventral axis. Spiking activity from position neurons was located with the guidance of three types of anatomical landmarks: morphological boundaries, vasculature, and fiber tracts (Fig. 4C). Activity was centered —1 mm rostral of the obex, 0.8 mm caudal of the caudal face of the facial lobe, and 0.4 mm lateral of midline; the center of area I in any given preparation could deviate by as much as 20% from these values. The midline bisector of two prominent blood vessels, one just caudal of the facial lobe and the other identified by its midline division into three or more branches, was within 250 μm of the center of position neuron activity. The center of activity was consistently 150–250 μm from the lateral edge of the visible medial longitudinal fasciculus (MLF). These indicators place area I in the vicinity of the border of rhombomeric segments 7 and 8. Typically, these indicators, taken together, allowed the identification of the center of area I within 5–10 exploratory penetrations.

Dye-labeling and histology

Neurons were filled with Neurobiotin (Vector Laboratories, Burlingame, CA) by iontophoresis with sharp intracellular electrodes or by use of the juxtacellular technique (Pinault 1996). Sharp electrodes (1.0 mm OD, 0.58 mm ID, borosilicate omega dot, AM Systems, Seattle, WA) used for intracellular dye injection were pulled on a horizontal laser puller (P-2000, Sutter Instruments, Novato, CA). Electrodes were first back-filled with dye solution (1–2% Neurobiotin, 2 M KCl, 10 mM Fast Green, pH = 7.4), then with normal intracellular recording solution (2 M KCl, 10 mM Fast Green, pH = 7.4), achieving a final resistance of 60–100 MΩ. Position neurons were penetrated with sinuoidal current bursts (0.5–2 ms, 2–5 kHz, 50% duty cycle) for 5–10 min. The amplitude of the injection current was attenuated if the width of the action potential increased.

Following a minimum 1-h period after Neurobiotin injection, goldfish were anesthetized with MS-222 and transcardially perfused with ACSF (with 1,000 U of heparin) followed by fixation solution (4% paraformaldehyde, 0.5% glutaraldehyde, 20 mM NaOH, in ACSF). Perfused brains were fixed overnight (4% paraformaldehyde), gelatin-embedded, and frozen. Sections (100 μm thick) were processed with the use of the avidin-biotin-peroxidase complex (Horikawa and Armstrong 1988). Neurobiotin label was revealed with dianaminobenzidine and NiCo histochemistry (Mesulam 1982). Mounted sections were counter-stained with cresyl violet. Tissue typically exhibited 10–20% shrinkage.

Experimental protocol

Twelve goldfish were used for the labeling studies. After extracellular localization of area I, penetrations aimed at sites of the highest density of position neurons were made with injection electrodes. To qualify for labeling, neural firing rate had to exhibit sharp transitions during saccades and a steady rate of activity during fixations, consistent with patterns of firing recorded extracellularly from position neurons (Fig. 1A). Only one position neuron in each half of the brain was injected. In some cases, 40 μg gallamine triethiodide (Flaxedil, American Cynamid, Wayne, NJ) or 1 μg doxacurium chloride (Neuromax, Glaxo Wellcome, Research Triangle Park, NC) was intramuscularly injected into the trunk after localization of area I to afford increased stability of recording. In these situations, attenuated eye movements could still be recorded. All Neurobiotin injections were performed in the light.

Six goldfish were used to develop a functional map of cell types in the medulla. After surgical exposure of the floor of fourth ventricle, recording penetrations spanning the depth of the brain stem were made in a region bounded rostrocaudally by the facial lobe and the obex, and mediolaterally by the MLF and the vagal lobes; penetrations were made at 100-μm intervals on a grid. In locations where position neurons were detected, penetration intervals were reduced to 25 μm. All of these experiments were performed in the light.

Fifteen other goldfish were used for the studies relating firing rates of position neurons to eye movements. Following area I localization, up to four recording sessions of different position units were performed in each fish, with each session lasting from 5 min to 2 h (total of 44 cells). All dark data were taken during the 10 min of dark immediately following a light period that was minimally 5 min long. In some cases, goldfish spent up to an hour in the dark during a session.

Analysis of eye motions and firing rates

SACCADE DETECTION. Time blocks containing saccades were determined by grouping together those time points for which the average eye velocity in the preceding 30-ms window matched or exceeded a threshold value of 10°/s. The leading edge of each time block defined the saccade onset time. During some saccades, a brief dip in eye velocity below the threshold level caused the algorithm to assign two time blocks to one saccade. Therefore onset times that were <250 ms after a preceding onset time were omitted from the detected set.

FIXATION PARAMETERS. Average firing rate and eye position was calculated over a 1-s window in each fixation beginning 0.5 s after saccade onset. Fixations of <1.6 s between saccades were excluded from study. To eliminate undetected saccades or significant respiration artifact, fixations were also excluded if the mean sum-squared deviation from the best-fit line to the calculation segment (all best-fit extracellular recording solution. After maximizing (to 1–2 mV) the size of the recorded action potential from a position neuron (with \( \pm 10 \) μm of lateral repositioning of the electrode when near the cell), units were dye injected with 2–50 nA of positive current (50 ms, 50% duty cycle) for 5–10 min. The amplitude of the injection current was attenuated if the width of the action potential increased.

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firing rate was calculated by taking the reciprocal of the average window by the duration of the window. In the second method, average calculated by dividing the number of spikes occurring within a given window. In the first method, average firing rate was calculated in one of three ways. In the first method, average firing rate was found by dividing the number of spikes occurring within a given window by the duration of the window. In the second method, average firing rate was found by finding the maximum rate within a 150-ms window centered on the moment of saccade onset. In the third method, the reciprocal of each element of the smoothed function was taken to produce an average firing rate function. The first method was the default, and the second one was employed when the expected spike count in a chosen calculation window was low. The third method was used when the rate function accompanying a sequence of saccades and fixations was of interest. The rate functions produced had sharper transitions associated with saccades than functions calculated by either of the other methods. The method used with a particular analysis is noted in the text.

LATENCIES. Separate algorithms, one for temporal and another for nasal saccades, were employed for the analysis of the latency between the onset of neural transitions and the onset of saccades. Transitions in firing rate associated with temporal saccades were analyzed within a window beginning 150 ms before and ending 50 ms after the moment of saccade onset. To minimize noise, transition onset times were only assigned if there was no spiking activity in the first half of the analysis window, the length of the first two ISIs in the second half of the analysis window was <20 ms, and the firing rate during the subsequent fixation was >10 spikes/s. For these cases, the time of the first spike within the second half of the analysis window was taken as the time of transition onset.

Transitions in firing rate associated with nasal saccades were analyzed within a window beginning 270 ms before and ending 100 ms after the moment of saccade onset. To reduce noise, a bias against small transitions was introduced by requiring that the average rate of firing in the initial 200 ms of the analysis window exceed 30 spikes/s (rate method 2). For the selected cases, the spikes in the latter 200 ms of the analysis window were used to determine the time of transition onset. This time was identified either by the location of the first spike followed by an ISI of >70 ms, or if none of the spikes had this property, by the location of the last spike if that spike was >70 ms from the end of the analysis window. If no spikes met these criteria, the saccade was ignored.

VELOCITY SENSITIVITY DURING SACCADES. The velocity sensitivity of position neurons during temporal saccades was assessed by analyzing the relationship between eye velocity and burst amplitude. Saccade velocity was measured by calculating the peak eye velocity in the initial 200 ms following saccade onset. Burst amplitude was assessed two ways: first by subtracting the presaccadic firing rate from the peak rate during a burst, and second by subtracting the postsaccadic firing rate. The peak firing rate during a burst was calculated by finding the maximum rate within a 150-ms window centered on the moment of saccade initiation (rate method 3, 50-ms convolution window). Only those saccades where the presaccadic firing rate was >5 spikes/s were included. Saccade velocity was only calculated for those cells for which more than eight data points were obtained.

RESULTS

The spontaneous eye movements of head-stabilized goldfish typically consist of a scanning pattern of horizontal saccades and fixations in which both eyes are directed toward one extreme, then span a range of 20 – 40° in two to four saccades to arrive at the other extreme (Easter 1971; Hermann and Constantine 1971). A segment of such a scanning pattern is shown in the eye-position records in Fig. 1A, accompanied by a single-unit recording from a representative position neuron from area I. The neuron progressed through a sequential set of nearly tonic levels of firing, one for each eye fixation. The onset of a temporal saccade (Fig. 1B) of the ipsilateral eye was preceded by the onset of an overshooting transition in firing rate that began with a burst of action potentials. Nasal saccades (Fig. 1C) were accompanied by undershooting transitions in the instantaneous ISI function for the spike train (in binwidths of at most 1.2 ms). This function was then convolved with a box-window, and the reciprocal of each element of the smoothed function was taken to produce an average firing rate function. The first method was the default, and the second one was employed when the expected spike count in a chosen calculation window was low. The third method was used when the rate function accompanying a sequence of saccades and fixations was of interest. The rate functions produced had sharper transitions associated with saccades than functions calculated by either of the other methods. The method used with a particular analysis is noted in the text.

FIG. 1. Activity of a position neuron during spontaneous saccades and fixations in the dark. A: horizontal eye position (top 2 traces), extracellular recording (middle), and firing rate (bottom) of an area I position neuron during a scanning pattern of horizontal eye movements. Position neurons transitioned rapidly between multiple states of steady firing. These persistent changes in the activity were accompanied by transitions of the eye from one fixation position to another. B: eye position and raster plot of action potentials for a temporal saccade. Bursts (underlined) of action potentials from burst-tonic position units preceded the onset of saccades (solid circle). For the unit shown, the onset time of bursts (dashed line) preceded saccade onsets by an average of 30.9 ms (SD) (Fig. 1A). In A, a 100-ms box window was used to calculate firing rate (rate method 3). The data in B and C are from nonsequential saccades of the same unit. Ipsil, ipsilateral; Contra, contralateral; T, temporal; N, nasal.
firing rate that began with a pause in action potential discharge. Following bursts, the rate of firing decayed to a new tonic level within 500 ms, and this new level of activity could be maintained with little decay or deviation. During the slide in firing rate, a slide in eye position to a new fixation value was frequently observed. This burst-tonic pattern was qualitatively similar to that observed during the discharge of abducens motoneurons (Pastor et al. 1991).

In the following, the results of labeling experiments will be presented that focused on outlining the dendritic tree and axonal projection patterns of position neurons. Next, results will be presented of a systematic exploration of the medulla in which neurons were sampled for several minutes to develop a functional map of cell types in the vicinity of area I. Following this, a quantitative analysis will be presented of position neuron discharge properties associated with changes in fixation position.

**Anatomy of position neurons**

Fifteen physiologically identified position neurons were injected with Neurobiotin using sharp intracellular microelectrode recording techniques or the juxtacellular labeling technique (Pinault 1996); nine were labeled and recovered for analysis (intra: \( n = 4 \), juxta: \( n = 5 \)). A representative example of changes in action potential firing associated with changes in fixation during an intracellular sharp microelectrode recording is shown in the inset of Fig. 2A. In all cases, discharge frequency could be modulated by passing current through the electrode (\(< 1.0 \, \text{nA} \) for intracellular, \(< 20 \, \text{nA} \) for juxtacellular, data not shown), indicating proximity to the action potential initiation site. In every preparation, only one labeled neuron was found within each area I. Camera lucida reconstructions in the coronal plane of two neurons are shown in Fig. 2, A and B. Photomicrographs illustrating morphological characteristics of position neuron soma, dendrites, and axonal projections are shown in the coronal sections of Fig. 3. In Fig. 2C, a schematic diagram in the horizontal plane of the brain stem is shown that depicts the axonal projections and primary dendritic field of position neurons determined from camera lucida reconstructions.

The somata of electrophysiologically-identified position neurons were fusiform in shape and \( \sim 20 \, \mu \text{m} \) in length (Fig. 3, A–D; \( n = 9/9 \)). All cell bodies were found 0.7–1.2 mm rostral of the obex, 280–420 \( \mu \text{m} \) from the midline, and 360–800 \( \mu \text{m} \) from the surface of the fourth ventricle. All somata were located within a column of the inferior reticular formation that was 100–150 \( \mu \text{m} \) wide and 200–300 \( \mu \text{m} \) thick (Fig. 3, A, B, and D, insets).
Two to three dendritic branches emanated from the somata of position neurons \((n = 9/9)\). As evident in Fig. 2, A and B, these dendrites branched repeatedly while ramifying over a wide extent of the medulla, primarily in the ventral region. The dendritic arbor extended from the midline to the entrance of the Xth nerve and from the level of the facial lobe \((1.2 \text{ mm rostral from the center of area I})\) to \(\sim 300 \mu \text{m rostral from the obex}\). Arborization was especially heavy in the first 500 \(\mu \text{m}\) rostral of cell somata (Fig. 2C).

In heavily labeled cells \((n = 4/9)\), spiny protrusions were visible on dendritic branchlets (Fig. 3, G and H, photomicrographs of the dendritic segments labeled G and H in Fig. 2B). These protrusions typically had shafts that were 2–4 \(\mu \text{m}\) in length, ending in \(\sim 1\mu \text{m}\)-sized swellings. At their highest density, spiny protrusions were spaced at intervals of \(\sim 5 \mu \text{m}\) (Fig. 3H).

Position neuron somata gave rise to a single axon hillock (Fig. 3, A–D). In six of nine cases, it was possible to follow the axon of the cell and determine its projection pattern. The hillock led to a sub-micron diameter axonal initial segment that was \(\sim 30 \mu \text{m}\) in length (Fig. 3C). Axons exhibited two types of projection patterns, one ipsilaterally directed and the other contralaterally directed.

In four ipsilateral cases, axons turned ventrally and then coursed rostrally, 300–500 \(\mu \text{m}\) from the midline, along a ventrally located fiber bundle (vfb) above the inferior olive (Fig. 2B). Axons could be followed at least up to the level of the facial lobe \((1.2 \text{ mm from the soma})\), where they began to overlap with the dendritic field of the caudal interneuronal group of the abducens (Fig. 2C). In two cases, the cell was labeled well enough to visualize axonal collaterals, with presynaptic boutons (Fig. 3, E and F), emanating from the parent axon at multiple levels through the abducens complex (Fig. 2C). In these cases, axons ventured as far as 500 \(\mu \text{m}\) beyond the rostral group of abducens neurons before either becoming too weakly labeled to follow or ending.

In two contralateral cases, axons projected toward the contralateral area I, crossing the midline beneath the MLF (Fig. 2A). In one case, the cell was labeled well enough to see that the crossing axon gave rise to collaterals with presynaptic boutons near the somata and proximal dendrites of contralateral position neurons (Fig. 2, A and C). Following this initial collateralization, the axon coursed rostrally along the ventral pathway described above, before collateralizing and terminating within the dendritic field of contralateral interneuronal neurons.

Axon collaterals and terminal arborizations were only visible in three of six cells with filled parent axons. Therefore the full extent of the axonal termination pattern of position neurons may have been underestimated.

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**Fig. 3.** Anatomy of position neurons. A–D: high-magnification coronal section photomicrographs of position neuron somata and proximal processes. Somata were \(\sim 20 \mu \text{m}\) in length, giving rise to 2 or 3 dendritic branches and 1 axon hillock (AxH). In C, the 2 segments of the axon hillock located on separate sections were reconstituted digitally, allowing visualization of the soma, axon hillock, initial segment (IS), and axon (Ax) simultaneously. The reconstruction of the cell in A is shown in Fig. 2B; the reconstruction of the cell in B is shown in Fig. 2A. Insets: low-magnification images of the goldfish medulla, with the locations of the labeled somata indicated by the boxes. Position neurons were located in a 100- to 150-\(\mu \text{m}\)-wide column of the inferior reticular formation. E and F: terminations at the level of the abducens (inset, F). G and H: spiny protrusions were seen on the dendrites of position neurons, with increased density of occurrence on more distal dendritic branches (H). X: Xth cranial nerve; m, midline; term, axon terminations; col, axon collaterals; VesC, vestibular commissure; Abd, abducens; VIn, abducens nerve; VII, facial nucleus; bp, boutons in passage; bt, boutons in termination; sp, spiny protrusions. Calibrations: A–D, 25 \(\mu \text{m}\); E–H, 10 \(\mu \text{m}\); insets, 100 \(\mu \text{m}\) except in A (1 \(\mu \text{m}\)).

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**Fig. 4.** Schematic diagram showing the localization of position neurons in the medulla of the goldfish. A and B: superpositions of action potentials from a triphasic unit and a biphasic unit. Biphasic responses were only seen in a restricted region (ellipsoid in C), while triphasic recordings were observed over a broader range of the medulla. C: area I, defined as the region in the inferior reticular formation containing position neuron somata, was located relative to morphological boundaries, vasculature (---), and fibers of passage (light gray). The probability of a biphasic recording from a position neuron with one electrode pass is illustrated by the size of the solid circles within the ellipse outlining area I (largest: \(>80\%), mid-sized: \(50\%\), smallest: \(<20\%)\). All features are drawn to scale. Distances are given in mm.

**Functional map of area I**

A systematic exploration of the medulla was made in six goldfish, spanning the area bounded by the vagal lobes, facial lobe, and obex (Fig. 4). In each fish, \(>20\) extracellular single-unit recordings were obtained from position neurons. Spiking activity from position neurons was separable into two distinct classes according to spike waveform: one class had triphasic action potentials (initially positive going, Fig. 4A), and the second had biphasic ones (initially negative going, Fig. 4B). Biphasic action potential waveforms were very stable; a recording from a single unit could be monitored over an electrode displacement of \(\sim 100 \mu \text{m}\) along the dorsoventral axis and maintained for several hours. Biphasic recordings were only seen in a restricted region that, as outlined in the following text, coincided with the locations of labeled position neu-
ron somata. Recordings of triphasic waveforms were unstable; recordings could only be monitored over distances of ~20 μm and were frequently associated with sudden wave-form amplitude changes (for example, a decrease in peak-to-peak amplitude from 0.8 to 0.3 mV). Triphasic waveforms were recorded over a wide range of the medulla that, as outlined in the following text, coincided with the range of the dendritic arbor of labeled position neurons. The stability and localization of biphasic waveforms indicate that they were recorded in proximity to somata, while the instability and widespread occurrence of triphasic waveforms indicate that they were recorded in proximity to processes.

The ellipse in Fig. 4C is centered at a location that corresponds to the approximate mean position of area I across the fish studied (anatomy is drawn to scale). As mentioned in methods, the center of position neuron (somatic) activity could deviate by as much as 20% from the average distances indicated. During vertical penetrations, the probability of encountering a position neuron soma was >80% in the caudal half of area I, ~50% in the rostral half, and <20% near the boundaries; this boundary scale is represented by the sizes of the filled circles in Fig. 4C. The encounter probability outside the bounding ellipse was <5%. The width of the ellipse along the mediolateral axes was ~125 μm, in close agreement with the width of the inferior reticular formation column in which labeled somata were identified. The length of the ellipse along the rostrocaudal axes was ~350 μm, consistent with the range over which labeled somata were found. Somatic recordings of position neurons in any given fish were encountered over a ~250 μm vertical range during penetrations, in close agreement with the thickness of the column in which labeled somata were identified. Across the population, this range was always encountered between the depths of 400–1,000 μm below the surface of the medulla. Therefore this functionally defined ellipsoidal region, ~350 μm in length, 125 μm in width, 250 μm in depth and coinciding closely with the location of position neuron somata, will be used to define the extent of area I.

On average, any given penetration in area I would yield approximately one encounter with a position neuron soma; at locations indicated by the largest circles, encounter rates could be as high as two position units every 100 μm. The lateral extent over which a somatic recording could be monitored was typically only 40 μm. This was established by obtaining a stable biphasic recording, retracting the electrode, moving laterally, advancing to record the same neuron (as determined by position sensitivity and onset threshold), and repeating these steps until spikes from the cell of interest were undetectable.

The likelihood of obtaining a triphasic recording from a position neuron process during a single penetration within the boundary of area I was ~20%. Encounter likelihood beyond this boundary was not the same in all directions: recording probability was ~20% during rostral penetrations and <5% during caudal penetrations. Also as penetrations further rostral were made, the mediolateral and dorsoventral range over which processes were encountered widened. A dramatic decrease in probability of encounter was observed beyond ~0.5 mm past the rostral border of area I.

The majority (>95%) of position neurons recorded had ipsilateral on directions, increasing firing rate with temporal (on) saccades and decreasing firing rate with nasal (off) saccades of the ipsilateral eye. Recordings of units with contralateral on directions were always of triphasic waveforms. This suggests that such recordings were from axons of position neurons with somata in the contralateral area I.

Approximately two-thirds of all single-unit recordings within the ellipsoidal boundary of area I were from position neurons. Four other cell types, with biphasic waveform recordings indicative of proximity to somata, were also encountered. One cell type was active in correlation with respiratory rhythms. This cell type was not correlated with eye movements when respiratory movements were not present. A second type was tonically active, not exceeding 1–2 spikes/s in discharge rate. A third type was clearly related to motions of the body and tail, discharging in bursts when the tail was flicked toward the ipsilateral side. The fourth type, encountered very infrequently (<2% of recordings) and not in every goldfish, exhibited a build-up in firing rate during a fixation, and, in contrast to position neurons, paused during temporally directed directed saccades. The first three cell types also composed the majority (>90%) of single-unit recordings obtained in the few hundred micrometers rostral or caudal of area I. In the regions within a few hundred micrometers medial or lateral of area I, very few recordings were indicative of proximity to a soma.

### Table 1. Fixation and saccade related parameters in the light and dark

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fixation</th>
<th>Saccade</th>
<th>Composite</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_h$</td>
<td>Mean $-15.1 [-2.1 -36.0]$</td>
<td>$-11.5 [1.8 -30.5]$</td>
<td>$-13.4 [1.2 -36.0]$</td>
</tr>
<tr>
<td>$k$</td>
<td>$2.5 [0.5 8.0]$</td>
<td>$3.0 [0.4 8.4]$</td>
<td>$2.8 [0.5 8.4]$</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>0.84, 17.7 [4.8 41.4]</td>
<td>0.86, 19.1 [5.9 56.8]</td>
<td>0.84, 20.5 [4.8 56.8]</td>
</tr>
<tr>
<td>$E_h$ vs. $k$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>33.4</td>
<td>24.6</td>
<td>28.3</td>
</tr>
<tr>
<td>$r$, $c$</td>
<td>0.65, (18.2 48.6)</td>
<td>0.55, (12.1 37.2)</td>
<td>0.65, (17.9 38.7)</td>
</tr>
<tr>
<td>$H$ vs. $k$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>6.0</td>
<td>9.2</td>
<td>5.7</td>
</tr>
<tr>
<td>$r$, $c$</td>
<td>0.93, (4.4 7.6)</td>
<td>0.90, (6.9 11.5)</td>
<td>0.90, (4.3 7.1)</td>
</tr>
<tr>
<td>$r$</td>
<td>Mean $0.24 [-0.08 0.81]$</td>
<td>0.21 (0.03 0.58)</td>
<td>0.24 [-0.08 0.81]</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>0.72</td>
<td>0.75</td>
<td>0.72</td>
</tr>
<tr>
<td>$L_m$</td>
<td>Mean $18.4 \pm 8.6$</td>
<td>$13.9 \pm 8.0$</td>
<td>$17.2 \pm 8.1$</td>
</tr>
<tr>
<td>$L_{10}$</td>
<td>Mean $22.0 \pm 21.2$</td>
<td>$24.0 \pm 20.7$</td>
<td>$21.8 \pm 19.9$</td>
</tr>
</tbody>
</table>

Values are means ± SD. Brackets enclose range over analyzed cells; underlines indicate average over analyzed cells. All $r$ values for measurements of position sensitivity were >0.6; all $r$ values for measurements of velocity sensitivity were >0.4. $E_h$: position threshold; $k$: position sensitivity; $E_h/k$: normalized position threshold; $\zeta$ : normalized position sensitivity; $H$: hysteresis; $r$: saccade velocity sensitivity measured by method 1; $r_2$: saccade velocity sensitivity measured by method 2; $L_m$: latency from burst to on saccade; $L_{10}$: latency from pause to off saccade; $r$: correlation coefficient; ci%: half of confidence interval as a percentage of the measured parameter; cl, confidence limits; * $P < 0.05$, † $P < 0.005$, ‡ $P < 0.001$. 

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consistent with area I residing along a column of the inferior reticular formation.

**Discharge properties**

To quantify saccade and fixation related discharge properties of position neurons, long-duration extracellular recordings were obtained from 44 neurons. Of these, 24 were recorded both in the light and dark, 5 were recorded only in the dark, and another 15 were recorded only in the light. Data were grouped into dark (n = 29), light (n = 39), and composite sets (n = 44; 29 dark and 15 light). Data, in both light and dark conditions, on position sensitivity, velocity sensitivity, latency to saccades, and the relation of position sensitivity to threshold of firing and hysteresis are summarized in Table 1.

The difference in means of the dark and light set population averages for position sensitivity, threshold, hysteresis, velocity sensitivity, and latency was not significant at the P < 0.05 level (t-test; used for all tests of difference in population means). Therefore summary information for the composite set is also provided in Table 1.

**Position sensitivity.** As shown in Fig. 1A, each new eye position after the occurrence of a saccade was accompanied by a different mean firing rate. For all 29 cells in the dark, fixation-averaged firing rate (rate method 2) and fixation-averaged horizontal position of the ipsilateral eye were well correlated above an eye-position threshold (mean r = 0.84, all r > 0.6, P < 0.005; 2-tailed Student’s t-test, used for all tests of correlation; fixations following on- and off-direction saccades over a period of 5–10 min were pooled). Representative data from two position cells in the dark are shown in Fig. 5, A and B. Light data for the cell of Fig. 5A is shown in Fig. 5C; note the qualitative similarity in slope and threshold. The best-fit lines determined for the population of neurons recorded in the dark are shown in Fig. 5D. For all cells in the dark, the slope (k) of the relationship between firing rate and eye position, a measure of the position sensitivity given in units of (spikes/s)/°, ranged from 0.5 to 8.0, with a mean of 2.5. The thresholds and slopes of cells were not topographically organized. All units in the light and dark had ipsilateral on direction, increasing firing rate for temporal saccades and decreasing firing rate for nasal saccades of the ipsilateral eye.

Position thresholds (E<sub>th,n</sub>) for the fits of firing rate to horizontal position of the ipsilateral eye were measured by determining the intersection of the best-fit lines like those in Fig. 5D with the abscissa. To facilitate comparison, eye position has been normalized by the oculomotor range of motion for each fish. As evident in Fig. 5D, all thresholds in the dark were restricted to the nasal half of the range of motion. Cells with lower normalized position sensitivities, k<sub>n</sub>, tended to have extreme nasal thresholds (Fig. 5D, inset). There was a significant correlation between the k<sub>n</sub> values and the normalized position thresholds, E<sub>th,n</sub> (r = 0.65, P < 0.001, slope = 33.4; uniform variance regression). For a goldfish with the typical range of motion of ±15°, this analysis indicates that an increase in slope of roughly 2 (spikes/s)/° is expected every 15° of threshold shift. The position sensitivities, thresholds, and correlation coefficients for the rate-position relationships were not significantly different between the light and dark conditions (P > 0.05).

**FIG. 5.** Quantitative analysis of the firing frequency of position neurons during spontaneous saccades and fixations. A and B: plots of fixation-averaged firing rate vs. fixation-averaged horizontal eye position for 2 different position neurons recorded in the dark. The regression of rate onto position for rate values >5 spikes/s was used to draw best-fit lines. For cell 1, the slope of the relationship, a measure of the position sensitivity, was 3.8 (spikes/s)/° (10 min of data, r = 0.91, P < 0.001), and the threshold (intercept with abscissa) was ~4.9°. For cell 2, the slope was 1.4 (spikes/s)/° (5 min of data, r = 0.96 and P < 0.001), and the threshold was ~21.5°. C: plot of fixation-averaged firing rate vs. fixation-averaged horizontal eye position for cell 1 when recorded in the light. The slope of the best-fit line was 3.5 (5 min of data, r = 0.94, P < 0.001), and the threshold was ~5.5, nearly identical to the values in the dark. D: the best-fit lines to the firing rate vs. eye-position relationships (normalized to the oculomotor range of motion for each fish) for the 29 cells recorded in the dark. The best-fit line for one unit was truncated at 100 spikes/s. The thresholds of all units were restricted to the nasal half of the field of motion, and, as seen in the inset, a correlation (r = 0.65, P < 0.001) was present between the slopes of the fits (normalized) and the normalized thresholds (the intercepts of the best-fit lines with the normalized abscissa). The extent of the error bars indicate 95% confidence intervals (c.i.) for the individual data points.
FIRING RATE HYSTERESIS. The firing rate following an on-saccade to a given eye position was typically less than that following an off-saccade to the same position. An example of this hysteresis in the relationship between firing rate and eye position is shown in Fig. 6A. In this figure for data from a single position neuron in the dark, \( F \) are from fixations following on saccades, and \( J \) from fixations following off saccades. The ordinate is the deviation in firing rate from the best-fit line of the rate-position relationship. As a measure of hysteresis, the difference in the means of on and off groups was calculated for data from the central 40% of fixations, where there was the highest degree of overlap between the groups. The hysteresis values in the dark for 13 analyzed cells (those units with 3 fixations in each group) ranged from 1.3 to 54 spikes/s (\( \bar{P} = 0.05 \), confidence limits of 0.5 and 4.3). B: plot of the difference in the means of the 2 groups (the hysteresis) vs. position sensitivity under dark conditions for 13 analyzed units; the extent of the error bars indicate the 95% confidence intervals for the individual data points. A regression of hysteresis onto position sensitivity reveals a strong correlation (\( r = 0.93, \bar{P} < 0.001 \)) with a slope of 6.0°.

VELOCITY SENSITIVITY. Position neurons exhibited burst-tonic behavior similar to that shown in Fig. 1. Typically, a burst of action potentials lasting no longer than 100 ms accompanied on-direction saccades, with the onset of bursts preceding saccadic motion of the eye (Fig. 1B). Burst amplitude was assessed two ways: first, by subtracting the presaccadic firing rate from the peak rate during a burst, and second, by subtracting the postsaccadic firing rate. The first method assumes that all of the burst is related to saccadic motion, while the second assumes that only the portion of the burst that is overshooting is related to saccadic motion. Forty-three units exhibited an overshoot in firing rate during on-saccades (mean value ranging from 2.7 to 54.6 spikes/s, population average = 26.0 spikes/s). One unit did not exhibit an overshoot (mean = −8.1 spikes/s).

The relationships between burst amplitude and saccade velocity in the dark for two representative neurons are shown in Fig. 7. Of 27 neurons from the dark set analyzed by the first
method, 24 had burst amplitude correlated with saccade velocity (mean $r = 0.72$, all $r > 0.4$, $P < 0.05$); in 3 cases, there was no significant correlation ($P > 0.05$). The slope ($r_1$, $r_2$) of this correlation, a measure of velocity sensitivity given in units of (spikes/s)/(°/s), ranged from $-0.08$ to $0.81$, with a mean of 0.24. Of 27 neurons from the dark set analyzed by the second method. 16 had burst amplitude only weakly correlated with saccade velocity (mean $r = 0.49$, all $r > 0.4$, $P < 0.05$); in 11 cases, there was no significant correlation ($P > 0.05$). The velocity sensitivities measured by the second method were on average 0.05, ranging from $-0.24$ to 0.36.

The relationship between velocity and position sensitivity was quantified for those neurons that exhibited a significant correlation between burst amplitude and saccade velocity ($n = 36$, composite set, method 1; $n = 22$, composite set, method 2). Velocity sensitivity increased with position sensitivity, with a correlation coefficient of 0.58 for data analyzed with method 1 (slope $=76 \pm 38$ ms, $P < 0.001$), and 0.57 for data analyzed with method 2 (slope $=72 \pm 48$ ms, $P < 0.005$).

**LATENCY.** The mean delay between the onset of a burst and the onset of a saccade for the population of units in the dark was $18.4 \pm 8.6$ ms (SD) ($n = 82$ bursts, $n = 9$ cells). The ranges of the mean and standard deviation for those units ($n = 9$) with at least three analyzed bursts were 6.2–24.1 and 1.3–11.2 ms, respectively.

Off-direction saccades were typically (>80% of the time) accompanied by undershoots in firing rate that began before saccade onset (Fig. 1C). The mean delay time from the last action potential before the beginning of this undershoot to the onset time of the saccade for the dark set was $22.0 \pm 21.2$ ms ($n = 165$ transitions, $n = 22$ cells). The ranges of the mean and standard deviation for those units ($n = 16$) with at least three analyzed off transitions were 11.3–45.7 and 8.7–34.6 ms, respectively. Relaxation to new tonic levels of firing occurred rapidly, within 500 ms.

One position neuron showed no burst activity associated with on-direction saccades. The first spike associated with a transition in firing rate accompanying an on-direction step in transition in firing rate accompanying an on-direction step in position neuron discharge observed during sinusoidal VOR stimuli (Pastor et al. 1994). Position neurons either had axons that followed a well-defined ventral pathway toward the ipsilateral abducens complex, or had axons that crossed the midline, projecting toward the contralateral group of position neurons and the contralateral abducens complex. Finally, the firing rate of position neurons was well correlated with horizontal position of the ipsilateral eye. These results provide evidence that area I position neurons are a central component of the horizontal VPNI.

One manner in which the role of area I in oculomotor behavior can be tested is through pharmacological inactivation experiments. Previous work has demonstrated that injection of lidocaine, a sodium-channel blocker, in a region including area I induced centripetally directed drift of the eye during fixations (Pastor et al. 1994). This result, by itself, shows that this region of the medulla may be part of the VPNI but does not distinguish between the role played by position neurons and other eye movement related neurons that may also have been inactivated. Evidence presented here indicates that the somata of position neurons in the medulla of the goldfish form a spatially localized cluster. Recordings from position neurons composed ~2/3 of those obtained from area I. No other cell type encountered in this region appears to be relevant to the issue of defining the role that this locus plays in oculomotor behavior: neurons with activity correlated to respiratory rhythms are likely to be involved in respiratory function, those related to tail motion are likely involved in axial movement, and those with low tonic firing rate do not characterize any known vestibular-related (Green et al. 1997) or optokinetic-related (Pastor et al. 1994) neurons. Neurons in which activity built-up during fixations were encountered too infrequently to play a significant part in defining the function of this region during normal saccades and fixations. Therefore in respect to the oculomotor system, area I can be viewed as a spatially segre-
gated locus that is composed almost exclusively of neurons carrying horizontal eye-position signals. This result, when coupled with results of inactivation experiments, provides further evidence that area I position neurons are elements of the goldfish VPNI for horizontal eye movements. A quantitative assessment of the precise contribution of these units to oculomotor function will require experiments in which the spatial extent of inactivation of area I and other medullary regions are monitored. Given the localization results presented here, such experiments can now be performed with a high degree of accuracy (Aksay et al. 1998).

There were no significant differences in the position sensitivities ($k$) or thresholds ($E_{th}$) of position neuron firing rates in the light versus in the dark (Table 1). Even though goldfish are afoveate, stabilization of the eye in the light is improved over the dark condition (Mensh et al. 1997). This improvement is likely due to the use of a retinal slip signal in a closed-loop control system that minimizes drift (Robinson 1981). If the feedback were to bypass area I, then the relationship between firing rate and eye position might be different in the light and the dark. Our finding of no difference between light and dark conditions is consistent with the idea that area I is part of the neural system that integrates these feedback signals to produce an improved signal for stabilization.

**Comparisons with mammalian position neurons**

The anatomy of position neurons in the goldfish most closely matches that of the “principal” cells in the NPH of the cat (McCrea and Baker 1985a,b). These cells, recorded in the anesthetized preparation, projected unilaterally and distributed terminal fields within the abducens complex, leading to the suggestion that this cell type generates the position signal recorded in the NPH in the awake preparation. The anatomy of position neurons in the goldfish did not match that of the “small” cells of the dorsolateral NPH in the cat, which had bilateral projections. Nor did it match that of the “multidendritic” cells of the caudal part of the cat NPH, which exhibited projections to the cerebellum. As described in previous work (Pastor et al. 1994), pressure injections of biocytin into the vestibulo-cerebellum of goldfish selectively labeled the inferior olive and brainstem area II, not cells in area I.

Field potentials recorded in the abducens nucleus of the cat following discharge of position neurons in the NPH are consistent with excitatory ipsilateral and inhibitory contralateral projections (Escudero and Delgado-Garcia 1988; Escudero et al. 1992). In support of the inhibitory nature of the contralateral projections, injection of tritiated glycine, a putative inhibitory transmitter in this system, into the abducens complex of cats resulted in label of the somata of neurons in the contralateral NPH (Spencer et al. 1989). The possibility that ipsilaterally and contralaterally projecting position neurons of goldfish have different physiological properties remains unexplored. Since all goldfish position neurons have ipsilateral on directions, the contralateral projection to the abducens would only make functional sense if it were inhibitory. A similar argument has been made for contralaterally projecting neurons in the primate NPH/MVN (McFarland and Fuchs 1992). Similarly, the ipsilateral projection from goldfish position neurons would only make functional sense if it were excitatory.

In this study, the term “position neuron” was used for any cell in area I whose tonic discharge during fixations correlated with eye position. In primate, two types of position neurons have been identified: those that were sensitive to eye-position, eye-velocity, and head-velocity, and those that were sensitive primarily to eye-position and -velocity only (Fukushima et al. 1992; McFarland and Fuchs 1992). The first type has been termed “eye/head-velocity” and the second “burst-tonic.” In cat, only position neurons that can be broadly termed “burst-tonic” have been described (reviewed in Fukushima et al. 1992). The contribution of head-velocity to the discharge of position neurons in cat and goldfish has not been determined.

The relationship between eye movements and neural activity for position neurons in area I was very similar to that for burst-tonic neurons in the MVN/NPH of cat (Delgado-Garcia et al. 1989; Lopez-Barneo et al. 1982) and monkey (Fukushima et al. 1992; McFarland and Fuchs 1992) under similar behavioral conditions (saccades and fixations). Units exhibited bursts of activity preceding the onset of on-direction saccades, relaxed to steady-state values of firing rate within 500 ms, and exhibited undershoots in firing rate accompanying saccades in the off direction. Cells were capable of maintaining steady firing during a fixation at values between 3 and 200 spikes/s. The position sensitivities of goldfish position neurons ranged from 0.5 to 8.4 (spikes/s)/°, comparable to those in the cat and monkey. Onset thresholds were distributed in the nasal half of the range of eye motion, and the relationship between position sensitivity and threshold indicated a recruitment order, again, in agreement with the trend in the cat and monkey. Such functional homology suggests that a common mechanism of integration may be used and that understanding the cellular and circuit mechanisms of the goldfish VPNI may generalize across vertebrate species.

Based on qualitative consideration of the saccade-related burst, the burst-tonic neurons of mammals have been separated into two or more groups: in primate, “burst-position” and “pure-position” (McFarland and Fuchs 1992), and in cat, “velocity-position,” “position-velocity,” and pure-position (Delgado-Garcia et al. 1989). Is it possible to assign area I burst-tonic neurons to similar subgroups? In primate, analysis of the latencies to on-direction saccades indicated that those neurons classified as pure-position generally began firing after the initiation of saccades, while those classified as burst-position generally began firing before. In goldfish, only one neuron was identified that consistently did not burst during saccades and began transitions in rate after saccades were initiated. Based on the low frequency of occurrence of this type of response in goldfish ($n = 1/44$), it is difficult to determine if pure-position neurons represent a distinct class or the tail end of a distribution. In the cat, it was noted that units were “distributed as a continuum in which a progressive decrease of eye-velocity sensitivity was accompanied by a proportional increase in eye-position sensitivity” (Delgado-Garcia et al. 1989). In the goldfish, however, there was a weak correlation between velocity and position sensitivities with a slope indicating direct proportionality. Thus it is inappropriate to attempt to divide these data into the same classes used in analysis of cat NPH/MVN neurons.
Area I projection to the abducens

Based on differences in the latencies of field potentials recorded in the abducens complex following position-velocity and pure-position neuron discharge in cat, it was hypothesized that pure-position neurons are the sole means by which position signals are supplied to the motoneurons (Escudero et al. 1992). For this hypothesis to be consistent across species, the activity of the injected area I neurons that projected to the abducens complex should have been characterized by a lack of burst activity during on-saccades. But this was not the case, as seen for a representative recording in Fig. 2A, where clear burst activity accompanied on-direction saccades. Furthermore it has been noted that in the monkey, the major output to the abducens is from the marginal zone of the NPH and that the majority of position neurons in the marginal zone are of the burst-tonic variety (Belknap and McCrea 1988; Langer et al. 1986). Therefore this hypotheses is either invalid or applies only to certain species.

Goldfish position neurons exhibited a significant rate-position relationship hysteresis associated with the direction of the preceding saccade; furthermore this hysteresis was correlated with the position sensitivity of the cell. The presence of hysteresis in motoneurons has been noted in previous work in the goldfish (Pastor et al. 1991), cat (Delgado-Garcia et al. 1986), and monkey (Goldstein and Robinson 1986). The origin of this hysteresis is unknown, but a significant muscle hysteresis may be a contributing factor (Collins et al. 1975; Goldberg et al. 1998). Abducens motoneurons of the goldfish were found to exhibit a hysteresis corresponding to a constant difference of ~10 spikes/s over most of the oculomotor range in the rate-position plots obtained after on- and off-directed saccades (Pastor et al. 1991). In the primate, abducens neurons (motoneurons and internuclear neurons) were reported to exhibit a significant hysteresis in their rate-position curves, corresponding to an average difference of 5.4 spikes/s near the middle of the range of eye motion (Goldstein and Robinson 1986). In those studies, no correlation was found between the static position sensitivity of an abducens neuron and its degree of hysteresis, in contrast with results presented here for position neurons. If the projection from the position neurons to the motoneurons was organized according to position sensitivity (high to high, low to low), then one would expect that the motoneurons would also have shown a correlation between hysteresis and sensitivity. Thus this difference is consistent with the idea that all abducens motoneurons receive the same position signal, a “common drive” derived from an average of the outputs of many position neurons. This would imply that the recruitment order seen at the level of the motoneurons (Pastor et al. 1991) is established independently of the recruitment order seen for position neurons.

The latencies reported here between saccade onset and firing rate transition onset for burst-tonic neurons were slightly greater than those reported for goldfish abducens motoneurons (Pastor et al. 1991) (17.2 ± 8.1 vs. 14.1 ± 4.8 ms for on-saccades, 21.8 ± 19.9 vs. 20.2 ± 5.6 ms for off-saccades). Assuming no offset was introduced by differing latency measurement methodologies, these results are consistent with bursts/pauses in position neurons contributing to the development of bursts/pauses in motoneurons. Furthermore firing rate bursts during on saccades were correlated with eye velocity, again, consistent with a role in the generation of saccades. However, during bilateral lidocaine inactivation of regions including area I (Pastor et al. 1994), large amplitude temporal and nasal saccades were still produced. This suggests that the burst activity of area I position neurons may not be a significant source of saccadic drive and that the parallel pathway of burst input to the motoneurons may be sufficient for the generation of saccadic motion.

What might be the primary function of position neuron bursts? One possibility is that they are necessary to produce the postburst slide in firing rate that was observed in goldfish area I position neurons. Similar postburst slides have been observed on the position neurons of the cat (Lopez-Barneo et al. 1982) and primate (McFarland and Fuchs 1992). Since burst neurons do not exhibit a slide, the slide in position neuron firing rate may serve as the primary determinant of the slide of motoneuron firing rates, a common feature in the abducens of goldfish (Pastor et al. 1991), cat (Delgado-Garcia et al. 1986), and monkey (Fuchs et al. 1988). This slide in motoneuron firing is thought to be important in overcoming the viscoelastic properties of the oculomotor plant during rapid eye movements (Goldstein and Robinson 1984; Optican and Miles 1985).

Implications for mechanisms of integration

The identification of position neuron axons projecting to the contralateral area I suggests a pattern of reciprocal connectivity, a form of recurrent synaptic drive. Since the on directions of area I cells in each half of the hindbrain are opposite to each other, it seems likely that this projection is inhibitory as was also suggested for the continuing projection of the main axon of these cells to the contralateral abducens. It is interesting to note that reciprocal inhibition can produce net positive feedback, a mechanism employed in network models of the VPNI (Cannon et al. 1983; Galiana and Outerbridge 1984). Such a substrate for feedback activity has been observed in the cat (McCrea and Baker 1985b; Spencer et al. 1989) and monkey (McCrea 1988). Preliminary efforts exploring the role of a reciprocal feedback pathway through midline-sections and unilateral inactivation led to the conclusion that a single intact area I could process position signals capable of driving both eyes (Pastor et al. 1994), although some eye movement deficits were observed after unilateral inactivation. However, the effects of these experiments were not studied in a quantitative manner, and therefore should be repeated in light of the results presented here.

The principal cells of cat NPH have axon collaterals within the nucleus (McCrea and Baker 1985a,b). Likewise, many cells in the NPH of the monkey also give rise to local collaterals (McCrea 1988). These local collaterals may serve as a means by which recurrent synaptic drive is produced among position neurons. Unfortunately, the results presented here do not contribute to an understanding of the presence or absence of local collaterals from area I position neurons in the goldfish because of incomplete labeling of the axonal process. It is likely that the Neurobiotin injections were at the soma or the large proximal dendrites. Given the small diameter of the axon initial segment and the injection location, it is possible that the flux of Neurobiotin to the axon was too small to significantly fill its collaterals and terminations. Also, very light labeling of spines on dendrites suggests the presence of diffusion barriers in these
neurons. Given the present identification of a ventral pathway by which the larger diameter distal axons course rostrally, an alternative future strategy to determine position neuron morphology could be to label through axonal microinjections, a strategy that has been successfully employed in the primate (Moschovakis 1997).

The region of heavy dendritic arborization rostral of cell somata closely coincided with a region in which extracellular recordings of triphasic action potentials were common. Since the extent of this region of triphasic signals was much greater than the narrow pathway that axons were seen to follow rostral of area I, it is likely that most of the processes from which the recordings were obtained were dendritic. Thus these results suggest that action potentials propagate along the dendrites of position neurons. Dendritic action potentials could activate dendro-dendritic chemical synapses mediating recurrent feedback among position neurons (Mori et al. 1982) or be involved with the gating of synaptic plasticity (Yuste and Tank 1996).

Neurons in the abducens complex of a teleost fish are electrically coupled through gap-junctions (Korn and Bennett 1975). In this study, intracellular microinjection of Neurobiotin produced at most only one labeled cell in each attempt. The absence of dye-coupling suggests that gap junctions are not present between position neurons in area I. Therefore it is likely any feedback between position neurons is not mediated through electrical synapses.

The threshold distribution pattern found in this study may have important consequences for the ability of the VPNI to hold the eyes at temporal positions. In a conductance-based recurrent-feedback model of the VPNI (Seung et al. 2000), it was observed that recruitment of new position neurons was necessary to compensate for the shortfall in synaptic feedback caused by synaptic saturation. In this model, a restriction of the onset thresholds to the nasal half of the range of eye motion resulted in a supra-linear increase in the magnitude of nasally directed drift with increasing temporal eye position. This pattern of drift closely matches what has been experimentally observed during spontaneous behavior in goldfish (Mensch et al. 1997).

The lateral range over which a position unit could be monitored was ~40 μm, and, on average, the number of units encountered on a single vertical penetration into area I was close to one. This, together with the overall size of area I shown in Fig. 4C, implies that there are ~30 position units contained within each area I. This number is comparable to the ~60 motoneurons in the abducens (Pastor et al. 1991) and the ~40 neurons in the medial rectus motor pool (Pastor et al. 1991). If area I provided all of the position signal driving fixations, then, on average, each position neuron would account for a few percent of the total. This raises the interesting possibility that lesion or stimulation of a single position neuron might have an experimentally detectable effect on oculomotor behavior.

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REFERENCES


ANATOMY AND DISCHARGE PROPERTIES OF POSITION NEURONS


