Anatomy and Physiology of the Primate Interstitial Nucleus of Cajal. II. Discharge Pattern of Single Efferent Fibers

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1 Department Basic Sciences, Faculty of Medicine, University of Crete, Crete, Greece 71110; 2 Institute of Applied and Computational Mathematics, Foundation for Research and Technology-Hellas, Crete, Greece 71110; 3 Eye and Ear Institute, Pittsburgh University, Pittsburgh, Pennsylvania 15213; and 4 Department of Otolaryngology, Washington University School of Medicine in St. Louis, St. Louis, Missouri 63110

Dalezios, Y., C. A. Scudder, S. M. Highstein, and A. K. Moschovakis. Anatomy and physiology of the primate interstitial nucleus of Cajal (NIC). II. Discharge pattern of single efferent fibers. J. Neurophysiol. 80: 3100–3111, 1998. Single efferent fibers of the interstitial nucleus of Cajal (NIC) were characterized physiologically and injected with biocytin in alert behaving monkeys. Quantitative analysis demonstrated that their discharge encodes a constellation of oculomotor variables. Tonic and phasic signals were related to vertical (up or down) eye position and saccades, respectively. Depending on how they encoded eye position, saccade velocity, saccade size, saccade duration, and smooth-pursuit eye velocity, fibers were characterized as regular or irregular, bi- or unidirectionally modulated, more or less sensitive, and reliable or unreliable. Further, fibers that did not burst for saccades (tonic) and fibers the eye-position and saccade-related signals of which increased in the same (in-phase) or in the opposite (anti-phase) directions were encountered. A continuum of discharge properties was the rule. We conclude that NIC efferent fibers send a combination of eye-position, saccade-, and smooth-pursuit-related signals, mixed in proportions that differ for different fibers, to targets of the vertical neural integrator such as extraocular motoneurons.

INTRODUCTION

The interstitial nucleus of Cajal (NIC) is the largest and most prominent of the cell groups of the medial longitudinal fasciculus (MLF). It contains at least two distinct cell classes, namely large pyramidal or multipolar neurons and small- to medium-size pyramidal, fusiform, or round cells (Zuk et al. 1982). Several lines of evidence have implicated the NIC in oculomotor control and in particular in the process of velocity to position integration in the vertical plane (for a review, see Moschovakis 1997). First, lesions of the NIC prevent monkeys and cats from holding eccentric gaze and impair their vertical vestibuloocular responses (Anderson et al. 1979; Crawford et al. 1991; Fukushima et al. 1992; Helmchen et al. 1998). Second, upward and downward medium lead burst neurons terminate profusely within the NIC (Moschovakis et al. 1990, 1991a,b). Finally, the discharge of NIC neurons often encodes the vertical position of the eyes (Fukushima et al. 1990; King and Leigh 1982; King et al. 1981).

Three projection systems are known to arise from the NIC (Kokkoroyannis et al. 1996): one directed through the posterior commissure that deploys dense terminal fields in the contralateral NIC, the oculomotor nucleus, and the trochlear nucleus, a descending system that deploys terminal fields in ipsilateral pontine and medullary nuclei and the ventral horn of cervical spinal segments, and an ascending system that deploys terminal fields in ipsilateral mesencephalic and diencephalic structures. The integrity of the first is crucial for normal velocity to position integration in the vertical plane because after lesions of the posterior commissure, the eyes can no longer be held at eccentric vertical eye positions (up or down) and the gain of the vertical vestibuloocular response (VOR) is reduced and its phase advanced, particularly at lower stimulation frequencies (Portasalis et al. 1994). If the vertical eye-position-related NIC signals are sent to the oculomotor complex via the posterior commissure, it should be possible to demonstrate the existence of fibers that originate in the NIC, course through the posterior commissure, and discharge in relation to vertical eye position. To test this, we studied the signals carried by oculomotor-related fibers in and near the posterior commissure of alert behaving squirrel monkeys and then injected these same fibers with a tracer to establish the location of their cell bodies and the targets of their axons. Here we describe the signals that were carried by such fibers. Preliminary versions of some of our results have appeared before (Moschovakis 1995; Moschovakis et al. 1997).

METHODS

Useful data were obtained from 14 adult squirrel monkeys of either sex, weighing 500–950 g. Animals were treated in accordance with the guidelines of the National Institutes of Health as stated in the Guide for the Care and Use of Laboratory Animals (DHEW Publication NIH85–23 1985) and with European Union directive 86/609 (Presidential Decree 160/1991). The methods employed have been extensively described before (Moschovakis et al. 1988, 1991a,b; Scudder et al. 1996a,b). Briefly, the animals were prepared for recording under sterile conditions and pentobarbital anesthesia (15 mg/kg). A stainless steel bolt was cemented on the occipital bone for head fixation, and preformed search coils, made of teflon-insulated stainless steel wire (Cooner), were sutured on the sclera of one or both eyes. In a second surgery 2 wk later, a parietal craniotomy was performed, and part of the cortex was aspirated to place a plastic chamber over the exposed left superior colliculus. The animals were alert and fully active within hours after surgery and showed no obvious neurological deficits.

Two days after surgery animals were placed in a primate chair with their heads fixed. Otherwise, they were free to move their body and limbs and appeared comfortable during the recording. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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sessions. Glass micropipettes filled with either a 10% solution of horseradish peroxidase or a 5% solution of biocytin in 0.5 M KCl and 100 mM tris(hydroxymethyl)aminomethane buffer (pH = 7.4), and beveled to <1-μm tip diameter (impedance: 40–80 MΩ) were inserted into the brain stem through the superior colliculus, and advanced toward the posterior commissure. Axon penetration was signaled by a 30- to 80-mV DC shift and the presence of 5- to 50-mV action potentials. We used the eye-coil method (Robinson 1963) to record the instantaneous position of the eyes with a resolution of 0.5°. Eye position was digitized on-line at a sampling rate of 2 ms, and the time between action potentials was measured with 100-μs resolution using the Spike2 software (Cambridge Electronics Design) running on a personal computer. Eye velocity was calculated off-line through software differentiation.

Digitized data were analyzed off-line. To evaluate the relationship between firing rate and eye position, we marked the beginning and the end of segments during which the eyes were fairly stationary (peak eye velocities <10°/s). These segments were ≥200 ms long and did not include pre- and postaccadic intervals. The computer stored the horizontal and vertical eye position at the beginning (H1 and V1) and at the end (H2 and V2) of the segment, the mean horizontal [H = (H1 + H2)/2] and vertical [V = (V1 + V2)/2] eye position as well as the intervals between all spikes within the segment. An estimate of the regularity of neuronal discharge was obtained from the coefficient of variation (CV = SD/mean) of interspike intervals for spikes emitted while the eyes were at or close to the primary position (within 2°).

To evaluate the relationship between parameters of the bursts of NIC fibers and parameters of saccades, we first marked the beginning and the end of saccades and the beginning and the end of related bursts. The computer then stored values for the following variables: saccade latency (Lat), saccade duration (Sd), burst duration (Bd), horizontal (ΔH) and vertical (ΔV) eye displacement, horizontal (VH) and vertical (VV) peak eye velocity, peak firing rate (Fm), and the number-of-spikes in the burst (Ns). Linear regressions between parameters of discharge and parameters of the movement included those between Ns and ΔV (ΔH), between Fm and VH (VH), and between Sd and Bd. To estimate the relationship between the firing rate of NIC fibers and the mean eye velocity during smooth-pursuit eye movements, we used a procedure previously employed by Skavenski and Robinson (1973). Briefly, we selected segments during which the monkey pursued smoothly an object of interest. For most neurons, we found several segments during which the eyes moved at average velocities up to ±60°/s. In these segments, we marked six interspike intervals symmetrically bracketing a certain eye position. The computer then stored the horizontal and vertical eye position that corresponded to the first (H1 and V1) and to the last (H2 and V2) spike, the mean horizontal ([H1 + H2]/2) and vertical ([V1 + V2]/2) eye position, the time of occurrence of the first (t1) and of the last (t2) spike, and the intervals between all seven spikes in the series. The average vertical (horizontal) eye velocity during such segments then was estimated as the ratio (V2 - V1)/(t2 - t1) ([V1 + V2]/2) (H1 - H2)/(t2 - t1). To estimate the average frequency of a neuron’s discharge associated with vertical (horizontal) eye velocity, we subtracted the firing rate attributed to the mean vertical (horizontal) eye position during the same segment (from the rate-position curve that had been established previously for the same fiber).

RESULTS

One hundred and forty-one oculomotor-related efferent fibers of the NIC were penetrated in and near the NIC and the PC of alert behaving squirrel monkeys. The intersaccadic discharge of 57 fibers was not modulated with eye position but rather was reminiscent of the upward medium lead burst neurons of the nucleus of the posterior commissure (Moschovakis et al. 1991a), of the downward medium lead burst neurons of the NIC (Moschovakis et al. 1991b), or of the long lead burst neurons located near the NIC (Scudder et al. 1996a). The remaining 84 fibers modulated their discharge in relation to eye position and are the topic of this report. Their intraxononal injection with a tracer enabled us to confirm the recording site of all fibers in our sample (27 in the PC and 57 in the NIC) and the origin and projection of many of them. The trajectory of their axons has not been completely reconstructed and will be the object of a future report. Preliminary results show that 39 of the 48 fibers recovered so far project through the PC, whereas the remainder (n = 9) descend via the MLF. The somata of all but 12 PC fibers were recovered, and their location in the NIC was ascertained. Because there were no differences between recovered and not recovered fibers and somata in terms of discharge pattern, we describe them together as a single group of NIC efferents.

Position-related discharge

The fiber illustrated in Fig. 1A displayed tonic intersaccadic activity and bursts that preceded saccades with an upward component, whether rightward or leftward. Figure 1B is a plot of mean intersaccadic vertical eye position (V; abscissa) versus mean interburst frequency of discharge (F; ordinate) for the same unit. The slope (3.5 spikes/s per degree of upward ocular deviation) and the correlation coefficient (r = 0.96) of the linear regression between the two variables are indicative of the sensitivity and of the reliability with which this fiber encoded the vertical position of the eyes. The intercept (102 spikes/s) is indicative of the unit’s intensity of discharge when the animal was looking straight ahead. The firing of this fiber was not correlated with the mean horizontal position of the eyes between saccades (right inset). To get an indication of the regularity of its tonic discharge during intersaccadic intervals, we determined the coefficient of variation (CV) of interspike intervals. Because the standard deviation of interspike intervals [SD(ISI)] increased in proportion to the mean ISI (Fig. 1B, left inset), we evaluated the CV for positions within 2° of primary position. Its value was low enough (0.06) to indicate that this unit was a regularly discharging one.

Other burst-tonic responses were much less precise. The fiber of Fig. 1C had a primary position firing rate of 66 spikes/s, increased its firing rate for downward eye positions, and decreased it for upward positions. The shallower slope of the rate-position curve (2 spikes s⁻¹ deg⁻¹) indicates that this fiber was less sensitive to vertical eye position than the one of Fig. 1B, whereas the large scatter of the data around the linear regression line indicates that this unit was less reliable in encoding the vertical position of the eyes (r = 0.67). The SD(ISI) of this unit obtained much higher values than that of Fig. 1B for roughly the same values of meanISI (inset). As a consequence, its CV was rather high (0.19), which underscores the irregularity of the tonic intersaccadic discharges of this fiber. As with the fiber of Fig. 1B, the firing rate of this unit was not correlated with the mean horizontal eye position (Fig. 1C, right inset).

The eye-position-related discharge shown in Fig. 1 is typi-
of the responses of 66 of the fibers we studied. The discharge of 35 units increased for upward eye positions, whereas the remaining (n = 31) increased their discharge for downward eye positions. Because vertical and horizontal eye position can covary due to sampling biases, we employed multiple regression to estimate each unit’s preferred direction (up or down, left, or right) as well as the slopes, intercepts, and correlation coefficients of their rate-position curves. Most of them (n = 61) modulated their tonic discharge only for vertical eye position. A small number of units (n = 5) had an oblique on-direction as indicated by the fact that their discharge was modulated for both vertical and horizontal eye position. Quantitative details of the relationship between the firing rate of NIC fibers and the vertical position of the eyes are summarized in Table 1. Figures 2A and 3A plot rate-position linear regressions for our 35 upward and 31 downward NIC efferents, respectively. In general, the two variables were well correlated for both upward (Fig. 2A, inset) and downward (Fig. 3A, inset) cells. The frequency histograms of the slopes of these relationships peaked at 4 spikes·s⁻¹·deg⁻¹ (Fig. 2B) and about −3.5 spikes·s⁻¹·deg⁻¹ (Fig. 3B) for upward and downward units, respectively, indicating similar sensitivities and considerable overlap (sign notwithstanding) for both types of fibers. Also, the frequency histogram of the primary position rate peaked at ~90 spikes/s for upward units (Fig. 2C) and between 70 and 80 spikes/s for downward units (Fig. 3C).

The slope of the rate-position curve and the primary position rate uniquely determine position threshold. In our sample, this was equal to −46.4 ± 52.7° (mean ± SD) for upward and 42.97 ± 45.6° for downward fibers, respectively. The majority of fibers were active well before primary position (Figs. 2D and 3D). When its whole range is considered, the position threshold (T) of NIC fibers is related to their vertical gain (k_v) through a power function of the form 48·T²ⁿ⁺¹ (r = 0.84; Fig. 2E) for upward cells and 28·T⁻⁰·⁷¹ (r = 0.9; Fig. 3E) for downward cells. To enable comparisons with previous samples, we also evaluated the relationship between vertical gain and position threshold when the latter is restricted to less than ±40°. In this case, the two variables are related linearly through an expression of the form k_v = 6.9 + 0.11T (r = 0.72) for upward cells and k_v = −4.7 + 0.05T (r = 0.45) for downward cells. Finally, few upward fibers had a CV >0.1 (Fig. 2F), and thus the population as a whole can be thought of as regular. Although a larger number of downward fibers had a CV >0.1 (Fig. 3F), on average these were no less regular than upward units (1-tailed t-test, t = −1.75, P < 0.1).

Other units (n = 12) modulated their discharge for upward but little or not at all for downward deviations of the eyes. Figure 4A illustrates the eye-position-related discharge of such a unit. As shown, upward deviation of the eyes was accompanied by increases in firing frequency; in contrast, the neuron did not fire less than ~75 spikes/s whatever the depression of the eyes. The same point is made by the plot of Fig. 4B, which illustrates the relationship between the mean intersaccadic vertical eye position (V) and the mean frequency of the same neuron’s discharge. There is an excellent linear relationship between the two variables when V is restricted to upward positions. However, this cell had a very small sensitivity (<10% of the on-hemifield) for downward eye positions (Fig. 4B). It is for this reason that we refer to fibers of this sort as “unidirectionally modulated.” Nor was the same unit’s tonic intersaccadic discharge related to horizontal eye position. The remaining six units modulated their discharge for downward but little or not at all for upward eye positions. Quantitative details of the relationship...
between the discharge of unidirectional fibers and eye position are summarized in Table 1.

**Saccade-related discharge**

In addition to eye position, NIC efferent fibers usually modulated their discharge for saccades. For example, the fiber of Fig. 5A emitted bursts the intensity of which varied in proportion to the size of upward saccades. In contrast, its discharge usually was depressed or even ceased for downward saccades. To evaluate whether parameters of this unit’s bursts were related to parameters of saccades, the number of spikes in the burst \( (N_s); \text{ordinate} \) was plotted against the size of the upward component of saccades \( (\Delta V); \text{abscissa} \) in Fig. 5B. There was an excellent linear relationship between the two variables \( (r = 0.97) \), whereas no relationship was found between \( N_s \) and the size of the horizontal component of saccades (Fig. 5B, inset). Further, an excellent corre-
Correlation (0.89) was found between the duration of this neuron’s bursts ($B_d$) and the duration of saccades ($S_d$; Fig. 5C). Finally, the peak frequency during bursts ($F_m$) was well related ($r = 0.93$) to the peak vertical velocity of accompanying saccades ($V_{\text{vert}}$; Fig. 5D).

As illustrated in Fig. 6A, the bursts that other fibers emitted for saccades were much less intense. Here again, burst intensity varied in proportion to the size of the upward component of saccades, whereas the discharge was depressed or ceased for downward saccades. The bursts of such fibers were less sensitive and often less precise in terms of the saccade metrics they encoded. Figure 6B plots the size of the upward component of saccades ($\Delta V$; abscissa) against the number of spikes in the bursts ($N_b$; ordinate) of the fiber illustrated in Fig. 6A. Although the two variables were well correlated ($r = 0.9$), the slope of the linear relationship is much more shallow (0.26 spikes/°). Here again, no relationship was found between $N_b$ and the size of the horizontal component of saccades (Fig. 6B, inset). The duration of this fiber’s bursts ($B_d$) was also well correlated ($r = 0.81$) to the duration of saccades ($S_d$; Fig. 6C). However, unlike the fiber of Fig. 5, modulation of the peak frequency ($F_m$) of its bursts could account for only ~15% of the variance ($r = 0.4$; Fig. 6D) of the peak velocity ($V_{\text{vert}}$) of the saccades they accompanied.

The saccade-related responses illustrated in Figs. 5 and 6 are typical of the pattern of discharge of the 65 burst-tonic units we encountered. Thirty-eight of them emitted bursts for upward saccades and 27 emitted bursts for downward saccades. Bursts preceded saccades by 4.3 ± 3.3 ms on the average ($n = 65$). In the 40 cells where pauses for off-direction saccades could be consistently documented, pause onset preceded saccade onset by 3.2 ± 3.7 ms on the average. Figures 7 and 8 provide summary illustrations of the relationships between saccade and burst parameters in upward (Fig. 7) and downward (Fig. 8) units, respectively. Figures 7A and 8A are cumulative plots of the 38 upward and the 23 downward linear regression lines between the number of spikes in the burst and the vertical size of saccades (up or down) that attained statistical significance ($P < 0.05$).
Similarly, Figs. 7B and 8B are cumulative plots of the 36 upward and the 27 downward statistically significant linear regression lines of burst duration versus saccade duration. The fact that they cluster around the diagonal indicates that burst duration was roughly equal to saccade duration for both upward and downward cells. Finally, Figs. 7C and 8C are cumulative plots of the 36 upward and the 16 downward statistically significant linear regression lines between peak firing rate and peak saccadic eye velocity. Frequency histograms of the slopes and correlation coefficients of these relationships are illustrated as insets in Figs. 7 and 8, whereas the range, average, and standard deviation of the values they obtain are summarized in Table 1. Note that relationships that did not attain statistical significance (P < 0.05) were eliminated from the population averages of Table 1.

It is important to note that the majority (n = 50) of the burst-tonic NIC fibers we encountered had the same on-direction for saccades and eye position. We refer to such units as in-phase units. Other fibers (n = 15) behaved quite differently in that they had opposite on-directions for saccades and eye position and are for this reason referred to as antiphase units. Six antiphase units emitted bursts for upward saccades but increased their discharge for downward saccade eye positions. Another nine units emitted bursts for downward
FIG. 7. A: linear regression lines describing the relationship between the number of spikes in the burst ($N_b$; ordinate) and the vertical size of saccades (abscissa) for 38 upward burst-tonic NIC units. B: linear regression lines describing the relationship between burst duration (ordinate) and saccade duration (abscissa) for 36 upward burst-tonic NIC fibers. Note that this plot contains fewer data points because the relationship did not attain statistical significance in 2 units. C: linear regression lines describing the relationship between peak rate of discharge (ordinate) and the peak vertical velocity of saccades (abscissa) for 36 upward burst-tonic NIC units. This plot also contains fewer points because the linear regression did not attain statistical significance in 2 units. Insets: frequency histograms of the slopes and the correlation coefficients of statistically significant relationships.

FIG. 8. Quantitative analysis of the saccade-related discharge of 27 downward burst-tonic NIC units. Plot layout as in Fig. 7.

saccades but increased their discharge for upward eye positions.

Figure 9, provides a third example of the saccade-related discharge we encountered among efferent fibers of the NIC. Fibers such as this ($n = 19$) neither burst nor paused for saccades and are for this reason referred to as tonic units. Nevertheless, tonic fibers are not as distinct from burst fibers as our name would imply. The saccade-related bursts of some burst-tonic neurons were small, whereas some tonic neurons emitted small bursts or paused for some saccades, particularly large ones.
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Vertical displacement of the eyes during saccades. Note that parameters of discharge differ from one NIC unit to the next, often significantly. Differences between units can be qualitative rather than quantitative. For example, \( \sim10\% \) of the units demonstrated some sensitivity to the horizontal position of the eyes. Also, \( \sim25\% \) of the units modulated their discharge in relation to upward or downward eye position, but not both. Finally, the discharge of \( \sim50\% \) of the fibers did not affect by smooth pursuit, whereas \( \sim25\% \) of the fibers did not emit any bursts for saccades.

Comparison with previous studies

The present study focuses on the output of the NIC, whereas previous work emphasized the response properties of cells located in the NIC regardless of whether they were projection neurons or not. NIC neurons previously were divided into burst-tonic (BT), irregular tonic (IrT), vestibular-and-saccade (VSN), and burst (BN) neurons in the monkey and into BT, VSN, and pitch neurons in the cat (reviewed in Moschovakis 1997). There are several reasons to think that it is the previously described BT neurons that the fibers of the present study resemble most. First, the tonic discharge of the fibers we encountered was well correlated with previous studies.

**Smooth-pursuit-related discharge**

To evaluate whether the discharge of efferent NIC fibers is related to smooth-pursuit eye movements, their firing rate was studied for short segments (lasting for \( \sim7 \) ISIs) during which the monkey smoothly pursued a target of interest (novel objects and morsels of food that were displaced slowly in front of its eyes). Figure 10 illustrates the relationship between the mean vertical velocity of the eyes (\( V_{v} \)) during such segments versus the mean firing rate (\( F \)) of a fiber after correcting for changes in firing rate that would be due to changes of eye position (from the rate-position curves of the same fibers). As shown in Fig. 10A, the two variables could be well correlated, in this case through the expression \( F = 0.64V_{v} - 2.99 \) \((r = 0.95)\). Enough data were available to carry out this analysis in 67 NIC efferent fibers (36 upward and 31 downward). Quantitative details about the relationship between the discharge of NIC efferents and the vertical velocity of the eyes during smooth pursuit are summarized in Table 1. About 60% of the fibers we examined (24 upward and 17 downward) modulated their discharge in concert with the vertical velocity of the eyes during smooth pursuit. Their on-direction for smooth-pursuit eye velocity was the same as their on-direction for eye position. The slope of the linear regression line could be as low as 0.3 (\(-0.1\) spikes/s per deg/s) or as high as 2.1 (\(-2.2\) spikes/s per deg/s) for upward (downward) fibers (Fig. 10B). Correlation coefficients between \( F \) and \( V_{v} \) ranged from 0.51 to 0.95 for upward units and from 0.44 to 0.97 for downward units (Fig. 10B, inset). The remaining fibers we tested (12 upward and 14 downward) did not modulate their discharge for vertical smooth-pursuit eye velocity.

**DISCUSSION**

The present study demonstrates that the firing rate (\( F \)) of efferent fibers of the NIC in alert, behaving monkeys is on the average related to the instantaneous vertical position (\( V \)) and the smooth-pursuit vertical velocity (\( V_{v} \)) of the eyes, through the expression

\[
F = 88 + 3.1 \cdot V + 0.8 \cdot V_{v} \tag{1}
\]

Additionally, NIC fibers emit \( \sim0.6 \) spikes per degree of vertical displacement of the eyes during saccades.
with the vertical position of the eyes during spontaneous saccades. Similar, excellent correlations between their tonic discharge and vertical eye position were described previously for BT neurons in both the rhesus monkey (King et al. 1981) and the cat (Fukushima et al. 1990). Further, the average vertical position sensitivity of the fibers we encountered (cf. Eq. 1) is quite similar to that of previously recorded NIC BT cells in rhesus monkeys (2.6 spikes s⁻¹ deg⁻¹) (King et al. 1981) and cats (3.9 spikes s⁻¹ deg⁻¹) (Fukushima et al. 1990). In contrast, primate IrT (King et al. 1981), primate VSN (Kaneko and Fukushima 1998), feline VSN (Fukushima et al. 1995), and feline pitch (Fukushima et al. 1990) neurons display little if any relationship with eye position, at least during spontaneous saccades.

Second, the NIC fibers we encountered discharged at a relatively high and regular rate when the animal was looking straight ahead. The average primary position firing rate of the fibers we encountered (cf. Eq. 1) agrees quite well with previous descriptions of BT NIC neurons of rhesus monkeys (79 spikes/s) (King et al. 1981) and cats (75 spikes/s) (Fukushima et al. 1990). In contrast feline pitch neurons (mean: 34 spikes/s; Fukushima et al. 1990) and VSNs (mean: 40 spikes/s; Fukushima et al. 1995) discharge at a lower rate. The same is true of primate IrT neurons (King et al. 1981). Also the units we studied discharged at a rather regular rate as indicated by their relatively low CV (mean: 0.09). Unfortunately, they cannot be compared with NIC neurons of the rhesus monkey because there is no information about the regularity of discharge of VSN, BT, and IrT cells in this species. On the other hand, the CV of the units we studied is similar to that of feline NIC BT neurons (mean: 0.15) (Fukushima et al. 1990). In contrast, feline pitch neurons (mean CV: 0.61) (Fukushima et al. 1990) and feline VSNs (mean CV: 0.5) (Fukushima et al. 1995) are quite irregular.

Finally, the majority of the NIC efferent fibers we encountered emitted bursts for vertical saccades. Consistent with previous descriptions of BT units in both the cat and the monkey, parameters of their bursts (number of spikes in the burst, duration, maximal rate of firing) were related to saccade parameters (saccade size, duration, and maximal eye velocity, respectively). BT neurons are not the only NIC cells that burst for saccades. More than half of the feline pitch cells also burst for saccades and quick phases (Fukushima et al. 1990) as do VSNs (Fukushima et al. 1995) in both the cat and the monkey (Kaneko and Fukushima 1998). However, the latencies of VSN bursts are in the long-lead range in both the cat (35 ± 14 ms) (Fukushima et al. 1995) and the monkey (32.5 ± 21 ms) (Kaneko and Fukushima 1998). They are thus much longer than the latencies of NIC BT units documented in this study (~4.0 ± 3.4 ms), a previous study in the rhesus monkey (~4.0 ± 2.5 ms) (King et al. 1981), and a previous study in the cat (~10 ± 3 ms) (Fukushima et al. 1990). To conclude, this extensive comparison indicates that the NIC efferent fibers we encountered correspond to the previously described BT neurons of the NIC and not to its pitch, VSN, or IrT cells.

Besides conventional, well-behaved, BT discharges, we observed a number of less conventional discharge types. First we observed that ~25% of the primate NIC efferent fibers encountered are not bidirectionally modulated with vertical eye position. This property was first described for pursuit neurons and ~30% of the vestibular-plus-eye-position neurons of the primate vestibular nuclei (Chubb et al. 1984). Similarly, many upward vestibular cells of the cat increase their rate with the upward deviation of the eyes and discharge at a constant rate for downward eye positions (Iwamoto et al. 1990a). Horizontal cells also can behave in a similar manner; the discharge of ~30% of the neurons of the primate nucleus prepositus hypoglossi level off for off-direction eye positions (McFarland and Fuchs 1992). Previous documentation of this phenomenon in NIC neurons has been limited (e.g., Fig. 3D of Fukushima et al. 1990). Moreover, some NIC fibers had their saccade- and position-related signals increase in opposite directions (antiphase units). The existence of antiphase neurons had been predicted by a model of the neural integrator (the so-called “rogue” cells) (Arnold and Robinson 1991) but previous documentation of discharge patterns such as this was limited to gaze velocity cells of the primate group Y (Tomlinson and Robinson 1984), eye and head velocity cells of the primate medial vestibular nucleus (Scudder and Fuchs 1992), and VSNs of the feline (Fukushima et al. 1995) and the primate (Kaneko and Fukushima 1998) NIC. The present study demonstrates that they are quite common among BT efferent fibers of the NIC (they amount to ~25% of the fibers in our sample).

To what extent can the discharge of vertical extraocular motoneurons be attributed to the input they receive from NIC fibers?

Assuming linear summation of inputs, a provisional answer can be had from comparisons between the discharge pattern of NIC efferent fibers and that of vertical motoneurons. In general, the rate-position curve of the NIC units we encountered is about two times shallower than that of vertical motoneurons (King et al. 1981; Robinson 1970). Unless the NIC output is amplified, some of the position sensitivity of vertical extraocular motoneurons must be due to sources other than the NIC, such as the vestibular nuclei (Chubb and Fuchs 1982; Iwamoto et al. 1990a, b; McCrea et al. 1987; Tomlinson and Robinson 1984). The importance of this additional input is shown by the fact that pontine MLF lesions interrupting the ascending projections of vertical secondary vestibular neurons cause vertical gaze nystagmus (Evinger et al. 1977).

The same is probably true of the bursts of NIC efferent fibers. Even when the sample is restricted to fibers that burst for saccades, these emit only ~0.6 spikes deg⁻¹ of vertical eye displacement, which is equal to about half of the saccadic sensitivity of vertical oculomotor neurons (Hepp et al. 1989). Again this implies that much of the burst of vertical extraocular motoneurons is due to sources other than the NIC, such as vertical medium lead burst neurons of the riMLF (King and Fuchs 1979; Moschovakis et al. 1991a, b). However, the fact that many NIC efferent fibers emit saccade-related bursts could explain why human subjects generate hypometric vertical saccades after lesions of the NIC (Fukushima 1991).

On the other hand, the slope of the rate-velocity curve of vertical oculomotor neurons (King et al. 1981) and the intensity and regularity of discharge of presumed primate oculomotor neurons (Robinson 1970) and trochlear motoneurons...
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FIG. 11. Cross-correlation between variables describing NIC unit discharge. Correlation coefficients ($r$) and $F$ ratios are shown for relationships which attained statistical significance ($P = 0.001$ or better). $k_v$, slope of the rate-position curve; $F_0$, firing rate at primary position; CV, coefficient of variation; Bi/Uni, bidirectional (Bi) or unidirectional (Uni) modulation of firing rate with vertical eye position; Lat, latency of saccade-related bursts; $R_V$, slope of the relationship between the number of spikes in the burst and the vertical displacement of the eyes; $r_{(Bd/Sd)}$, correlation coefficient of the relationship between burst duration and saccade duration; $G_{(Fm/Vm)}$, slope of the relationship between maximal burst frequency and maximal saccadic eye velocity; $\Delta \Phi$, neuron classes in which tonic and burst discharges increase in the same ($\Delta \Phi = 0^\circ$) or in the opposite ($\Delta \Phi = 180^\circ$) directions.

(Fuchs and Luschei 1971) when the eyes are at primary position is similar to that of NIC efferent fibers. Accordingly, these aspects of motoneuronal discharge could be considerably influenced by the input they receive from the NIC.

Are there subpopulations of NIC efferent fibers?

Much of this study is devoted to a systematic exploration of a large number of parameters describing the discharge pattern of NIC efferent fibers. Each one of these parameters can be thought of as an axis of a multidimensional space, and every fiber in our sample can be described by its location in this space. The question we wish to consider is whether the units we studied fall into clusters corresponding to functionally distinct groups of neurons. To reduce the dimensions of the parameter space, we employed a principal component analysis. Figure 11 illustrates the cross-correlation between the nine variables we studied and the results we obtained. Each one of its boxes shows the location of each one of the units encountered in the plane formed by two of the variables studied. When one variable was discrete, such as in-phase/antiphase, we used analysis of variance (ANOVA) to compare the two classes in terms of the continuous variable. In the case where both variables were discrete, we used a $\chi^2$ test.

Examination of Fig. 11 shows that several correlations were significant. Burst latency (Lat) and $R_V$ (the slope of the relation between vertical size and the number of spikes in the burst) are related to several other variables. We found...
that the earlier the onset of the bursts, the higher the fidelity with which they encoded saccade duration ($r_{(E/S)}$, row 6), the higher the slope between their maximal frequency and the maximal saccadic velocity ($G_{(F/M)}$, row 7), and the higher the $R_v$ (row 5). Also, fibers emitting weaker bursts (small $R_v$) were more irregular (high $CV$, column 3). This is consistent with a previous description of feline NIC BT units (Fukushima et al. 1990). Moreover, the higher the $R_v$ (column 6), the higher the fidelity with which the bursts of NIC efferent fibers encoded saccade duration and the higher the slope between their maximal frequency and the maximal velocity of saccades. The bursts of bidirectional units had longer latencies than those of unidirectional units (column 4), whereas antiphase units discharged more irregularly (higher $CV$) and emitted weaker bursts (shallower $R_v$) than in-phase units (row 8). Finally, the more reliably a neuron’s bursts encoded saccade duration the higher the sensitivity with which they encoded the maximal vertical velocity of saccades (column 7). With the exception of the good correlation between $F_0$ and $CV$, all other correlations were not significant. Units which did not modulate their discharge for saccades (tonic neurons) were analyzed separately. An ANOVA did not reveal differences between tonic and BT neurons in terms of regularity of discharge, firing rate at primary position, and rate-position slope. The monkey vertical neural integrator therefore differs from the cat horizontal neural integrator, the tonic neurons of which are thought to be more regular than BT neurons (Escudero et al. 1992).

For our principal component analysis, we elected to exclude the regression coefficients of the rate-velocity, rate-position, and $N_b$ versus size curves as well as the gain of the curve relating burst duration with saccade duration from our cross-correlation analysis because the high correlations between the slopes and the correlation coefficients of these analyses rendered them redundant. We also did not include any of the smooth-pursuit variables because vertical smooth-pursuit eye velocity was not related to the discharge of $\sim 50\%$ of the units in our sample. Suffice it to say that sensitivity to vertical smooth-pursuit eye velocity was not correlated with any of the nine discharge parameters of Fig. 11. Finally, we restricted the parameter space to continuous variables and excluded units that had a zero value in any one of these variables. Following these restrictions, there were 45 BT units left in our sample; three factors were determined that accounted for almost 79% of the variance in this considerably restricted sample. The first factor (accounting for $\sim 45\%$ of the variance) was related to parameters of saccade-related bursts (i.e., latency, slope of the relationship between $N_b$ and saccade size, regression coefficient of the relationship between burst duration and saccade duration, and slope of the relationship between maximal frequency and maximal saccadic velocity). The second factor (accounting for $\sim 20\%$ of the variance) was related to the regularity and intensity of discharge at primary position. The third factor (accounting for $\sim 14\%$ of the variance) was related to position sensitivity. These three factors ("burstiness," "regularity," "sensitivity") form the axes of the three-dimensional plot of Fig. 12. As shown here, NIC efferent fibers form a diffuse cloud one end of which is made of regular but relatively insensitive units that emit strong bursts. The cloud continues through units of average regularity and sensitivity that also emit fairly strong bursts and ends with units that emit weak bursts and vary in terms of regularity and sensitivity. Although the absence of bursts did not allow us to extend the principal component analysis to tonic NIC units, these can be thought of as a continuation of the same cloud on a plane orthogonal to the "burstiness" axis and located some distance away from its origin (such as the regularity-sensitivity plane that forms the floor of Fig. 12).

To summarize, the efferent fibers of the NIC encode a constellation of oculomotor variables in terms of a complex array of discharge parameters. Each of these variables has enough spread to serve as a basis for separating the units into classes, but whatever the variable chosen, the gulf between the extremes always is occupied by fibers of intermediate properties. Even using parameters of discharge that are inherently dichotomous to divide the population of NIC efferent fibers into bi- and unidirectionally modulated units or into in-phase and antiphase units does not break the NIC efferent fibers into meaningful functionally distinct groups because neither bidirectional nor antiphase units differ in other respects much from units with antithetical properties. Therefore we conclude that NIC efferent fibers occupy a functional continuum in the parameter space that defines their discharge. Further we conclude that each NIC efferent fiber sends a combination of eye-position, saccade-, and smooth-pursuit-related signals to targets of the vertical neural integrator (including extraocular motoneurons). To determine whether identical copies of these signals are sent simultaneously to all targets of the NIC, we must know the trajectories and patterns of termination of functionally identified axons that arise from individual NIC neurons. Their study will be the object of a future report.
REFERENCES


