Characteristics of the Pupillary Light Reflex in the Macaque Monkey: Discharge Patterns of Pretectal Neurons

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Pong, Milton and Albert F. Fuchs. Characteristics of the pupillary light reflex in the macaque monkey: discharge patterns of pretectal neurons. J Neurophysiol 84: 964–974, 2000. Anatomical and physiological data have implicated the pretectal olivary nucleus (PON) as the midbrain relay for the pupillary light reflex in a variety of species. To determine the nature of the discharge of pretectal light reflex relay neurons, we recorded their activity in monkeys that were fixating a stationary spot while a full-field random-dot stimulus was flashed on for 1 s. Based on their discharge patterns, neurons in or near the PON came in two varieties. The most prevalent neuron discharged a burst of spikes 56 ms (on average) after the light came on followed by a sustained rate for the duration of the stimulus (burst-sustained neurons). When the light went off, nearly all neurons (33/34) ceased firing, and then all the neurons with a resting response in the dark (n = 15) resumed firing. Both the firing rate within the burst and the sustained discharge rate increased with log light intensity and the latency of the burst decreased. The burst and cessation of firing were better aligned with the stimulus occurrence than with the onset of pupillary constriction or dilation. Taken together, these data suggest that burst-sustained neurons respond to the visual stimulus eliciting the pupillary change rather than dictating the metrics of the subsequent pupillary response. Electrical stimulation at the sites of four of five burst-sustained neurons elicited pupillary constriction at low stimulus strengths after a latency of ~100 ms. When the electrode was moved 250 μm away from the burst-sustained neuron, the elicited response disappeared. Reconstructions of the locations of burst-sustained lumiance neurons place them in the PON or its immediate vicinity. We suggest that PON burst-sustained neurons constitute the pretectal relay for the pupillary light reflex. A minority of our recorded pretectal neurons discharged a burst of spikes at both light onset and light offset. For most of these transient neurons, neither the burst rate nor the interburst rate was significantly related to light intensity. We conclude that these neurons are not involved in the light reflex but subserve some other pretectal function.

INTRODUCTION

Studies to understand the neural substrate of the human light reflex have been limited to clinical cases of patients with neural lesions that affect the pupil (see Johnson 1983 and Thompson 1992 for review). Because the neural discharge that controls the reflex is not accessible in human subjects, it needs to be inferred from studies of other species. The pupillary light reflex of nonhuman primates is very similar to that of humans (Pong and Fuchs 2000), making it a suitable model for studies of the neuronal basis of the reflex.

Anatomical studies in monkeys have implicated the pretectal olivary nucleus (PON) in the dorsolateral mesencephalon as the first relay in the pupillary light reflex. Tritiated amino acids injected into the vitreous chamber of the eye and absorbed by the retina produced orthograde labeling in both PONs as well as in the sublenticular region of the pretectum (Benevento et al. 1977; Dineen and Hendrickson 1983; Hendrickson et al. 1970; Hutchins and Weber 1985; Pierson and Carpenter 1974). Stimulation in the monkey pretectum elicited pupillary constriction (Magoun et al. 1936), and bilateral lesions of the monkey PON abolished the light reflex (Carpenter and Pierson 1973). In turn, the PON projects bilaterally to the Edinger-Westphal (EW) nucleus, which lies anterior and dorsal to the oculomotor complex. After the EW was filled with horseradish peroxidase (HRP), retrogradely labeled cells were found in both PONs (Büttner-Ennever et al. 1996; Steiger and Büttner-Ennever 1979). After the PON was filled with tritiated amino acids, orthograde label appeared in the lateral visceral cell column of the EW nucleus, dorsal to the oculomotor nucleus, bilaterally (Benevento et al. 1977; Büttner-Ennever et al. 1996). Warwick (1954), using retrograde degeneration, was the first to identify the EW nucleus as the preganglionic nucleus for the pupil. Fills of the ciliary ganglion with wheat-germ agglutinated HRP retrogradely marked the ipsilateral EW nucleus (Akert et al. 1980; Burde and Loewy 1980). These experiments suggest that the most direct pathway for the pupillary light reflex in the monkey is the four-neuron arc schematized in Fig. 1.

In a variety of mammals, the firing of PON neurons has been shown to increase with the intensity of the light stimulus. In cats, most of the PON luminance neurons could be activated antidromically from the EW nucleus at latencies of 1.5–3 ms (Distler and Hoffmann 1989). The pretectal neurons in the cat displayed a burst-sustained pattern of discharge in response to light onset and a pause in response to light offset (Sillito 1969). Some pretectal neurons produced only a transient discharge at light onset and offset. They were recorded dorsal to the PON and could not be activated antidromically from the EW nucleus (Distler and Hoffmann 1989). In rats, PON neurons also increased their discharge rates with luminance and both tonic-on and transient cells were encountered (Clarke and Ikeda 1985;
FIG. 1. Schematic of the direct pathway of the pupillary light reflex. Each retina projects bilaterally to the pretectal olivary nucleus (PON). Each PON then projects bilaterally to the Edinger-Westphal (EW) nucleus. Each EW nucleus projects to the ipsilateral ciliary ganglion, which then projects to the pupillary sphincter muscle in the iris. See text for a complete description.

METHODS

General procedures

The subjects in these experiments were three juvenile male rhesus macaques (Macaca mulatta) weighing 2.5–3 kg. Eye movements were monitored by the scleral search coil technique, whose characteristics have been amply documented in other publications from this laboratory (e.g., Fuchs et al. 1993). The animals were rewarded for aiming their eyes within ±2° of a small laser spot projected on a screen before them. During elicitation of the pupillary light reflex, the monkeys were required to hold their eyes steady by fixating a stationary spot. To elicit eye movements to test the behavior of other pretectal neurons, we moved the spot by galvanometer-mounted mirrors that intercepted the spot on its way to the screen. The control signal for the galvanometers came either from an Apple Macintosh IIx computer, which caused the spot to move in jumps to elicit saccadic eye movements, or a function generator, which moved the spot in sine waves to elicit smooth-pursuit eye movements.

Tungsten electrodes (0.005-in diam), coated with a specially formulated epoxy resin and polyimide tubing and exposed by 10 μm at the tip, were advanced through the monkey brain by a hydraulic microdrive (Trent Wells). The microdrive was mounted on a stainless steel recording chamber attached to the monkey’s skull and directed at the pretectal olivary nucleus. The center of the chamber was inclined by 20° to the sagittal plane and aimed 8 mm dorsal and 1 mm anterior to ear-bar 0 and 2 mm lateral to the midline. The chamber was positioned over a 30-mm hole trephined in the monkey’s skull. The implantation of the coil, stabilization mounds to hold the head and the recording chamber was performed under strictly aseptic conditions while the monkey was under deep anesthesia (Fuchs et al. 1993).

The conditions under which these experiments were performed complied with National Institutes of Health standards as stated in the “Guide for the Care and Use of Laboratory Animals” (Department of Heath Education and Welfare Publication NIH85-23 1985), Institutional Animal Care and Use Committee recommendations at the University of Washington, and the American Association for Accreditation of Laboratory Animal Care.

Search strategy

As the electrode was advanced ventrally through the brain, we employed three search conditions. In the first, we elicited visual responses by a 1-s projection of a slide of randomly spaced 1-cm² black squares, distributed uniformly over the entire screen. The luminance of the white areas on the screen measured 4 cd/m². This pattern illuminated the complete visual field of the animal and thus provided a large stimulus to elicit the light reflex. In this condition we recorded not only unit activity but also pupillary area, as measured by the pupillometer described in the companion paper (Pong and Fuchs 2000). The animal’s left pupil was monitored in all sessions. As will be seen in later figures, the random-square pattern elicited a brisk pupillary light reflex.

Ideally we would have used the same single LED stimulus that elicited pupillary constriction in the previous study. Initially, however, we could not find the neurons with the LED alone, probably as will be seen later, because the neurons were localized to a very small area. Therefore we switched to the much brighter larger field stimulus. After recording responses from several neurons with the large field stimulus, we simply continued using it for consistency. In addition, the patterned stimulus, when moved, allowed us to evaluate the possible motion sensitivity of PON neurons and drive other known motion-sensitive neurons in the pretectum (see next paragraph). In the second condition, the monkeys tracked a smoothly moving laser spot, oscillating at 0.5 Hz, ±10°. The smooth-pursuit tracking was used to identify neurons of the nucleus of the optic tract (NOT) (Mustari and Fuchs 1990), which discharge for image motion across the retina during ipsiversive smooth pursuit. In the third condition, when we encountered neurons with pausing in their discharge during saccades, we rewarded the monkey for tracking target jumps of ±5, ±10, and ±15°. Pauses in discharge after saccades in any direction and amplitude are characteristic of pretectal following omni-directional pause neurons (FOPN) (Mustari et al. 1997).

Neurons whose discharge was related to the visual stimuli while the monkey fixated the stationary laser spot were subjected to a variety of stimulus luminances. After ±10 blink-free responses were collected at the brightest intensity, the luminance of the patterned stimulus was gradually reduced by a series of neutral density filters in the projection path of the stimulus. The amount of light entering each eye was always measured at several intensity attenuations ranging from 0.4 to 3 log units. At each intensity, stimuli were presented once every 3 or 4 s. The attenuation of light intensity was continued until the neuron no longer responded to the flashes as judged by broadcasting the neural activity through an audio amplifier. If the unit was still
isolated after the entire range of filters had been employed, the patterned slide was replaced with a clear slide and the attenuating filters were removed to create a more intense stimulus. Under these conditions, the luminance was 8 cd/m². The electronic signal that opened and closed the projection shutter was recorded to indicate the onset and offset of the light stimulus.

In our first subject, monkey E, we recorded eye-movement-related neurons under conditions that drove them most vigorously to use the location of these previously described units (Mustari and Fuchs 1990; Mustari et al. 1997) to help locate the PON. Eye-movement-related neurons in monkeys S and R were noted but not recorded.

**Electrical stimulation**

Electrical stimulation was applied at some recording sites by passing pulse trains (biphasic 100–µs pulses at 300 Hz and 20–100 µA for 1–2 s) through the recording electrode. The current was generated by a Nuclear Chicago stimulator (Model 7150). The actual current strength was probably less than that set on the stimulator and cited in a Nuclear Chicago stimulator (Model 7150). The actual current

1–2 s) through the recording electrode. The current was generated by a Nuclear Chicago stimulator (Model 7150). The actual current strength was probably less than that set on the stimulator and cited in the text because of likely loss due to capacitance coupling in the cable to the animal.

**Locating the recording sites**

In the last week of recording, marking lesions were placed in each monkey by the passage of ~30 µA of positive DC current for 30 s through the tip of the electrode when it was positioned at particularly fruitful loci. The monkeys then were killed and perfused transcardially with a saline wash followed by 10% formalin. Frozen 40–µm sections in the frontal plane were mounted and counter-stained with cresyl violet. Representative electrode tracks were reconstructed, guided by the marking lesions.

**Data analysis**

The horizontal and vertical eye and target positions, the pupillary area, and a signal indicating light onset and offset were recorded on magnetic tape with the use of a pulse-code modulation VCR system (Vetter) or FM recorder (Honeywell 5600). The eye, target, and pupil signals were digitized at 1 kHz by a National Instruments A/D conversion board connected to an Apple Macintosh IIx computer. The time between action potentials was determined with an interrupt conversion board connected to an Apple Macintosh IIx computer.

**RESULTS**

**General properties of neuron responses**

We recorded the activity of 66 neurons that responded to changes in luminance (“luminance neurons”) in the pretectum of three monkeys: 40 in monkey S, 20 in monkey E, and 6 in monkey T. To describe the features of the whole population, we tested all the neurons with a standard stimulus consisting of a nonattenuated, full-field projection of a random-square pattern flashed for ~1 s.

Two different types of luminance neurons were found in the vicinity of the PON. In response to the random-dot pattern, one type (n = 54) discharged an initial burst of spikes and then maintained a lower level of firing above the prestimulus level while the light was on, i.e., they exhibited a burst-sustained pattern of discharge (Fig. 2A). These burst-sustained cells continued to fire steadily after the pupil had stabilized at its final size. Like the neuron in Fig. 2A, the majority of these neurons (n = 31) did not discharge during the interval between stimulus presentations when the background was either dark or very dimly lit. The remaining burst-sustained neurons (n = 23) briefly ceased firing (i.e., paused) when the pattern was turned off and then resumed their prestimulus rate.

The second type of pretectal neuron encountered (n = 12) displayed a burst of firing at both light on and off (Fig. 2B). Whereas some of these transient cells did have a sustained discharge, it was their burst at light off that distinguished them from burst-sustained neurons. Unlike the burst-sustained neurons, 11 of the 12 transient neurons discharged with light off. The remaining neuron fired a burst only at light on and did not have a sustained discharge.

To be used in further quantitative analysis, a neuron had to show ≥10 responses, free of blinks and eye movements, to the standard stimulus. Thirty-four of the 54 burst-sustained neurons and 11 of the 12 transient neurons met this criterion. The relative proportions of the two types were roughly similar in the three monkeys.

**Burst-sustained neurons**

**RESPONSE TIMING TO STIMULUS AND PUPILLARY CONSTRUCTION.** To determine whether the discharge of burst-sustained neurons was better synchronized with the visual stimulus or the pupillary response, we aligned the neuronal discharge rasters and their histogram with either the onset of the light stimulus or the start of pupillary constriction. An example of such a comparison is shown in Fig. 3. The onset of the burst and the end of the
sustained response both were better timed with the onset of the light stimulus (Fig. 3A) than with the onset of pupillary constriction (Fig. 3B). To provide a quantitative assessment for all of the neurons, we compared the standard deviation of the average latency from the flash of the full-field random-square pattern to burst onset with the standard deviation of the average latency of the burst to the onset of pupillary constriction (Fig. 4). For all but four neurons, the data lay below or on the line with unity slope; thus the onset of the burst relative to pupillary constriction was at least as variable as, and occasionally more variable than, the timing between the onset of the stimulus and the burst.

In response to the standard stimulus, the burst started at a mean latency of 56.3 ± 19.3 ms (range: 43–62 ms) across the three monkeys. It lasted for an average duration of 94.4 ± 45.5 ms (range: 84–154 ms), and its mean discharge rate averaged 92 imp/s (range: 82–142 imp/s). The longer durations occurred in monkey T, whose bursts consisted of two successive peaks. After the burst there was a sustained discharge with a mean...
discharge averaging 30 imp/s (range: 26–52 imp/s) across the three monkeys.

For both the burst and the subsequent sustained discharge, the average firing rate increased with stimulus luminance for all neurons as illustrated for the representative neuron in Fig. 5. Also, as stimulus intensity increased, burst latency decreased.

RELATION OF FIRING TO LIGHT INTENSITY. The relation of firing rate to stimulus luminance is shown in Fig. 6. The data points are for individual trials from the unit whose behavior is illustrated in Figs. 3 and 5. The response of each cell was fit with a log-linear regression for either the burst (Fig. 6A) or the sustained (Fig. 6B) rate. The fit for the data points is shown as a thick line. Both the average burst and average sustained rates of 19 of 24 neurons showed a significant increase with stimulus luminance ($P < 0.01$). For two units, only the burst relation showed a significant increase and for two others only the sustained rate did. The correlation coefficients ($r$) ranged from 0.36 to 0.88 (mean = 0.61) for the burst and from 0.31 to 0.94 (mean = 0.75) for the sustained rate ($P < 0.01$). The slope for the burst rates ranged from 6 to 49 imp/s/log luminance (mean = 29) and the slope for the sustained rates ranged from 1.4 to 40 imp/s/log luminance (mean = 11). A plot of the slopes of the burst-luminance and the sustained rate-luminance relations for those 19 units where both relations were significant ($P < 0.01$) also are shown. Burst duration was taken as the period of increased firing above the baseline and the subsequent sustained level. The average burst rate was calculated as the number of spikes in the burst divided by burst duration.

RELATION OF BURST TIMING TO LUMINANCE. The average time from light onset to burst onset, i.e., burst latency, decreased with increasing light luminance. The relations of burst latency with stimulus luminance were fit with log-linear regression lines (Fig. 7A). Eighteen of the 24 neurons tested displayed negative slopes ranging from $-14$ to $-87$ ms/log luminance (mean = $-31$) with $r$ ranging from $-0.33$ to $-0.93$ (mean = $-0.57$; $P < 0.01$). To the standard full-field stimulus (Fig. 7B), the burst latency was $56.3 \pm 19.3$ (SD) ms across all 34 units from the three monkeys. Burst duration was significantly related to stimulus luminance for only 11 of 24 neurons tested ($P < 0.01$).

RELATIONS TO PUPILLARY MOVEMENT METRICS. The average burst and sustained firing rates were correlated with constriction amplitude and peak constriction velocity for a minority of the units after we removed the influence of luminance by calculating a partial correlation coefficient with luminance fixed (Snedecor and Cochran 1967). Eight of 24 units tested showed a significant correlation ($P < 0.05$) between average burst rate and constriction amplitude. Eight showed a significant correlation ($P < 0.05$) between average sustained rate and constriction velocity.
DISCHARGE PATTERNS TO UNUSUAL CONSTRICTIONS. The time course of pupillary constriction in monkey S became biphasic (Fig. 3B, open arrow) after we began recording in its left pretectum. There were two successive constrictions with the first about half that of the maximum. The maximum constriction amplitude was not different before and after left side recordings, but the constriction duration became nearly two times longer for the same amplitude: average constriction duration in response to the standard stimulus was $462.8 \pm 47.9$ ms for the seven neurons on the left side and $870.9 \pm 75.8$ ms for the last 18 units on the right. The constriction durations during recordings from the left side were comparable to those in the other two animals ($T$: $436.8 \pm 38.7$ ms, $n = 5$ neurons; $E$: $515.4 \pm 224.6$ ms, $n = 4$). The latency from the light stimulus to pupillary constriction also was different during left- and right-side recording: $133.8 \pm 9.5$ ms during left-side recording and $158.9 \pm 9.6$ ms during right-side recording. Again, the latency while recording on the left was more like the averages for the other two animals ($T$: $144.4 \pm 12.5$ ms, $E$: $127.5 \pm 9.7$ ms).

The neural firing patterns of the burst-sustained neurons recorded in monkey S did not reflect this alteration in the time course of the pupillary constriction (Fig. 3). Burst discharge frequencies and durations were not different ($P < 0.05$) for units recorded on the left and right sides nor were the burst latencies or the sustained rates. These results suggest that the change observed in pupillary constriction would be reflected at a site downstream from the pretectal neurons being studied. Furthermore these results, like those in Fig. 3, show that PON neural discharge reflects light stimulus properties more closely than the metrics of the resulting pupillary constriction.

**Transient Discharge Patterns to Unusual Constrictions.**
Eleven transient neurons fired bursts of spikes when the random-square pattern was turned on or off (Fig. 2B). The bursts followed either light onset ($n = 1$) or offset ($n = 1$) or both ($n = 9$) and preceded the start of the pupillary response to the change in the light stimulus. Standard deviations of the average times from the onset of the full-field stimulus to the burst were less than those of the average times from burst to pupillary constriction but the difference was not as great as for the burst-sustained neurons. Like the neuron of Fig. 8, 9 of the 10 transient neurons displayed two successive short bursts when the pattern was turned on; 2 of 10 with a burst when the pattern turned off had double bursts. None of the nine were from monkey T, in which the burst for the burst-sustained neurons sometimes also exhibited a double burst. On average,
the second burst had half the frequency of the initial burst and followed it by ∼50 ms.

Between the bursts for light onset and offset, 6 of the 11 transient neurons were active. In response to the standard stimulus, the discharge rates during this inter-burst interval averaged 19.8 ± 6.8 imp/s. On average, the burst at light onset \((n = 10 \text{ neurons})\) had a latency of 51.9 ± 20.1 ms and lasted 69.3 ± 29.5 ms, with an average discharge rate of 136 ± 50.3 imp/s. The burst at light offset started 83.2 ± 53.4 ms after the light offset and lasted for 99.1 ± 43.5 ms, with an average discharge rate of 100.6 ± 43.7 imp/s.

For only one of five neurons tested at different intensities did the burst discharge change significantly \((P < 0.01)\) with stimulus luminance. Similarly, the latency from stimulus onset to the burst decreased with luminance in only one of the five transient neurons. The burst with light offset also did not change with stimulus luminance. It showed much greater variability in average burst rate from one intensity to the next than did the burst at pattern onset. Of the four neurons with interburst discharge that were tested at different luminances, only two showed significant relations of interburst firing with luminance \((P < 0.01); one showed an increase and the other a decrease. For example, the interburst firing of the unit in Fig. 2B was not significantly related to light intensity.

Effects of electrical stimulation

To test whether the pupillary light reflex was mediated by neurons in this pretectal region, we injected current through the recording electrode at the site of luminance neurons in one monkey. The stimulation site was in the right pretectum of monkey \(S\) and its left pupil was monitored. Stimulation caused a clear pupillary constriction at four of the five sites of burst-sustained luminance neurons at stimulus strengths of <50 μA (Fig. 9). On tracks through three of those sites, stimulation was applied at different depths around the luminance neuron. In two of the tracks, stimulation only in the immediate vicinity of the luminance neuron elicited constriction. On one track, stimulation 500 μm either dorsal or ventral to the neuron elicited no response and on the other stimulation 250 μm ventral to the neuron elicited no response. On the third track, responses still could be elicited 850 μm dorsal to the recorded luminance neuron but only at a 67% higher stimulation intensity than that required at the site of the luminance neuron. At three of the four sites of a luminance neuron, the pupil constricted only transiently on stimulation and then dilated back to the prestimulus size before stimulation ended. This pattern occurred at the site illustrated in Fig. 9. At the site of another burst-sustained luminance neuron, the pupil constricted and then dilated to an intermediate area, which was maintained until the stimulation ceased. At the two sites that were tested (at both, stimulation elicited the lowest threshold response at the site of a luminance neuron), the amount of constriction increased and the latency decreased with increasing stimulus strength. The minimum constriction latency averaged 112.4 ± 27.3 ms across the four sites.

In the companion paper, we showed a strong linear correlation between the peak constriction velocity and the change in pupillary area (Pong and Fuchs 2000). A similar linear relation obtained when the constriction was caused by electrical stimulation. For the combined data obtained during stimulations along two different tracks, the slope of the relation was 4.89 mm²/s/mm² with a correlation coefficient of 0.76 \((n = 112 \text{ responses})\). This slope, obtained in monkey \(S\), compares favorably to the slopes produced when pupillary constriction was elicited by light stimuli in three other monkeys in the companion paper.
ion study (Pong and Fuchs 2000); the relations for those monkeys, \( R, E, \) and \( T \), were 5.79, 4.96 and 5.84 \( \text{mm}^2/\text{s/mm}^2 \), respectively. The similarity of the peak velocity-area relations in the stimulation and natural situations shows that the relation still obtains when the discharge delivered from the PON is a presumptive simple 1- to 2-s train. The stimulation experiments therefore imply that the relation between peak velocity and area is likely to be a property of the pupillary plant rather than the exact patterns of innervation that drive the sphincter muscle.

**Location of luminance neurons**

Most of the luminance neurons were isolated in the immediate vicinity of the PON, the oval-shaped nucleus indicated by arrows in the frontal section through the mesencephalon shown in Fig. 10. In our three monkeys, the PON averaged 0.42 ± 0.045 mm in depth (dorsal-ventral), 0.94 ± 0.13 mm in width (medial-lateral), and 0.50 ± 0.061 mm in length (anterior-posterior), illustrating the small size of our target.

Electrolytic marking lesions along fruitful recording tracks (Fig. 10) indicated that the luminance neurons were at the same depth as the PON in all three monkeys. In the majority of the fruitful tracks (42/54), only one luminance neuron was found. In the remaining 12 tracks, multiple luminance neurons were encountered but over an average dorsal to ventral extent of only 0.27 ± 0.23 mm.

The depth of the luminance neurons was also confirmed by the consistent pattern of neural discharge above and below them. Neurons lying immediately dorsal to the luminance neurons displayed a strong sensitivity to ipsiversive visual slip or image motion across the retina. Neurons with these characteristics have been identified as residing in the NOT (Hoffmann and Schoppmann 1981; Mustari and Fuchs 1990), part of which lies just dorsal to the PON (Mustari et al. 1994). Neurons dorsal to the visual slip neurons paused in their discharge after the onset of saccades in all directions. These cells, which have been called FOPN, have been localized to a thin layer dorsal to the NOT (Mustari et al. 1997). Ventral to the luminance neurons, we occasionally found cells that paused for saccades, a firing pattern that is characteristic of fixation cells in the rostral superior colliculus (Munoz and Wurtz 1992).

In the rostral-caudal (AP) and medial-lateral (ML) dimensions, the location of the luminance neurons and the reconstructed histological location of the PON exhibited considerable overlap. The AP-ML location of the luminance neurons along the horizontal plane relative to the PON was calculated from the placement of the electrode tracks and marking lesions within the recording cylinder and the position of PON boundaries relative to the recovered lesions. Based on these determinations, luminance neurons were placed relative to the boundaries of the PON. Forty-one of the 45 luminance neurons with ≥10 recorded responses lay within 1.5 mm of the center of the PON. The luminance neurons were encountered over an average extent of 1.39 ± 0.73 mm ML and 0.87 ± 0.42 mm AP across the three monkeys. Therefore the volume of the brain space occupied by the luminance neurons had the same size and shape as that occupied by the PON as estimated from the histology.

**DISCUSSION**

We have shown that there are two types of neuron in or near the macaque PON, and they respond to visual stimuli with different discharge patterns. The majority discharge a burst of spikes when the light is turned on and continue with a lower sustained discharge while light intensity is maintained. The others discharge a burst of spikes when the light is turned on and/or off and revert to lower rates in between.

In the following sections, we will consider a number of issues concerning the two types of pretectal neurons. First, is their discharge related solely to the visual stimuli or is there a motor component to their discharge? Second, what is the significance of their firing patterns? Third, do our data help us decide whether one or both groups might participate in the pupillary light reflex?

**PON neurons do not discharge with pupillary constriction per se**

Although pupillary constriction occurred at a relatively fixed latency after presentation of the patterned stimulus, we nevertheless could demonstrate that the unit response was better timed with the stimulus than with a change in pupillary size. For burst-sustained neurons, the change of both the burst and pause in activity was clearly more abrupt when firing was aligned on the time that the stimulus was turned either on or off than when firing was aligned on the onset of constriction or dilation (Fig. 3). Furthermore, only a minority of neurons had significant relations between average burst and sustained discharge rates and also constriction amplitude and peak constriction velocity. Nor was there a relation between burst duration and either peak constriction velocity or change in constriction amplitude. For transient neurons, the timing of the burst also was better with stimulus onset than with the start of pupillary constriction, but this difference was not so clear.
In monkey S, repeated penetrations in the vicinity of the PON caused pupillary responses to become almost twice as long as normal (872 vs. 462 ms) with prominent double humps in their velocity profiles. However, the neural firing pattern of the burst-sustained neurons did not reflect these changes in pupillary dynamics (Fig. 3). These observations support the conclusion of our timing analysis that PON neural discharge reflects light stimulus properties more closely than it does the metrics of pupillary constriction. Also the change in the dynamics of pupillary constriction inadvertently produced in monkey S must have been the result of activity produced downstream from the pretectal neurons being studied.

Eight of 24 burst-sustained neurons tested at different luminances exhibited a significant correlation \( (P < 0.05) \) between average burst discharge rate and constriction amplitude after the effects of luminance were factored out. Similarly, 8 of the same 24 burst-sustained neurons had significant correlations between average sustained rate and constriction amplitude. Seven of the 24 neurons displayed a significant correlation between average burst discharge rate and peak constriction velocity. These observations suggest that the discharge of these neurons potentially was contributing to the signal that constricted the pupil. However, because only a minority of the 24 neurons sampled exhibited these relationships, it may be that not all PON neurons provide the same signals for pupillary constriction.

### Luminance neuron firing patterns

For the burst-sustained neurons, not only is the timing of the discharge best related to the stimulus, but the metrics of their discharge patterns also vary with stimulus intensity. Both the transient and sustained firing rates increased and the burst latency decreased with the log of stimulus luminance. In an earlier study, Gamlin et al. (1995) also found that PON neurons discharged with a burst-sustained firing pattern to a brief light stimulus. Our data agree well with theirs, which also showed a linear increase in steady firing with log light intensity. The slopes of the relations that we measured from their figures \((n = 16 \text{ neurons})\) averaged 7.5 imp/s/log luminance, whereas in our study the slopes averaged 29 imp/s/log luminance \((n = 21)\). One explanation for the difference is that Gamlin et al. (1995) used a circular stimulus that subtended a visual angle of \(\pm 18^\circ\), whereas we used a significantly larger, rectangular full-field stimulus that subtended \(\pm 36^\circ\) horizontally and \(\pm 26^\circ\) vertically. Although our stimulus did consist of a random-square pattern, it probably illuminated a larger extent of the retina.

In contrast to the burst-sustained neurons, the transient neurons did not exhibit a consistent change in firing with stimulus luminance. For the on response, burst frequency changed with luminance for only one of five neurons tested, and the latency of the burst decreased with luminance for only one of the five neurons. The off-response burst did not change with luminance for any of the transient neurons.

Does our observation that the firing of PON cells is primarily visual account for the burst-sustained discharge pattern? As the pupil constricts, less light would fall on the retina and pretectal visual neurons should undergo a decrease in firing. However, a close look at Figs. 2 and 3 reveals that the burst is over and firing has dropped to postburst rates before pupillary constriction even begins. Therefore the burst-sustained discharge pattern is the result of the input signals to PON cells and/or their intrinsic membrane properties. Consistent with our argument is the finding that PON neurons still exhibit a burst-sustained discharge when the visual stimulus is delivered in a Maxwellian view to cause an “open loop” pupillary constriction (Gamlin et al. 1995).

Although the metrics of the burst are not related to the dynamics of pupillary constriction, the burst-sustained firing of PON neurons clearly provides a pulse-step change in firing to its target EW neurons. A similar burst-step firing is required by oculomotor neurons to overcome the viscosity of the extraocular muscles and the orbital mechanics. Perhaps the burst-sustained discharge pattern during constriction and the pause-sustained activity during dilation helps to jump-start the response of the smooth muscle which constricts the pupil. The peak constriction velocity occurs in the first third of the constriction (Pong and Fuchs 2000), so a high initial drive seems necessary.

In the companion paper, we commented on the variability in monkey pupillary responses to the same stimulus luminance (Pong and Fuchs 2000). The diversity in the details of the response patterns for the individual trials illustrated in Figs. 2, 3, and 5 shows there is a variability in the neural response as well. Thus one cause for the variability in pupillary constriction is likely to be the variation in the neural signal sent downstream to the sphincter muscle.

### Are burst-sustained luminance neurons part of the pupillary light reflex?

To deal with this question, we must consider several points. First, anatomical evidence from several species suggests that the midbrain relay for the pupillary light reflex is the PON. In particular, injections of HRP into the EW nucleus of monkeys produced retrograde labeling of PON neurons (Steiger and Büttner-Ennever 1979). Also, bilateral lesions that included the PON abolished the light reflex in monkeys (Carpenter and Priester 1973). In monkey S, repeated penetrations into one pretectum caused alterations of the response dynamics of the pupil. This unfortunate occurrence suggests that electrode tracks into the region of the PON damaged part of the circuitry involved in producing normal pupillary constriction.

We have already demonstrated that the firing of our pretectal burst-sustained neurons is related to stimulus luminance, and therefore they have discharge characteristics that would be appropriate for participation in the light reflex. In the next sections we consider anatomical, electrophysiological, and stimulation evidence that they participate in the pupillary light reflex.

### Localization based on anatomical reconstruction

Because both the area containing our luminance neurons and that constituting the PON are very small, the placement of our luminance neurons relative to the PON has been problematic. Because the PON is so small, we chose to make our marking lesions after we had exhausted all of the productive tracks, by which time the nucleus may have shifted position slightly relative to our coordinate system due to brain swelling, for example. Nevertheless, we were able to estimate the size of our productive recording area as \(0.39 \pm 0.73\) mm medial-lateral, \(0.87 \pm 0.42\) mm anterior-posterior, and only \(0.27 \pm 0.23\) mm dorsal to ventral. These dimensions compare favorably with...
the limits of burst-sustained luminance neurons in the monkey PON, namely, 1,000 um medial-lateral, 500 um anterior-posterior, and 300 um dorsoventral (Gamlin et al. 1995). Based on marking lesions such as those in Fig. 10, 41 of the 45 luminance neurons with ≥10 recorded responses lay within 1.5 mm of the center of the PON. It should be pointed out that the somata of PON neurons tend to be located in the shell of the nucleus (Sun and May 1995) at distances from the center of the PON where many of our burst-sustained neurons were recorded.

**Localization based on nearby unit activity.** To further help localize our luminance units, we documented the location of eye-movement-related units known to lie in their immediate vicinity. In particular, nearby units that respond during full-field visual stimuli and smooth pursuit have been recorded from the NOT (Hoffmann and Distler 1989; Mustari and Fuchs 1990), which lies just dorsal to the PON. Also, neurons that pause after saccades in all directions are reliably encountered in a very thin band just dorsal to NOT neurons (Mustari et al. 1997). Because eye-movement-related neurons were often found on the same penetration as the more ventral luminance neurons, we confidently conclude that luminance neurons were consistently encountered at the same depth as the PON. Although we are not absolutely certain that every luminance neuron was in the PON, the combined evidence strongly indicates that most were.

Although the rostral pole of the superior colliculus (SC) lies at the same anterior-posterior level as the luminance cells we reported here, we have several reasons to believe that they did not lie in the SC. First, many rostral SC cells discharge a burst with small saccades (Munoz and Wurtz 1992) and none of our cells nor any in the vicinity did. Second, none of our neurons paused in their firing for saccades as do rostral SC neurons (Munoz and Wurtz 1995). Finally, when on several tracks we drove beyond the area that yielded our neurons, we encountered units with discharge characteristics typical of SC neurons. Therefore our visual neurons appear to lie ≥1 mm dorsal to the SC.

**Effects of electrical stimulation.** The results of electrical stimulation at the sites of luminance neurons further support our suggestion that they participate in the pupillary light reflex. At the sites of four of five of our luminance neurons, relatively weak stimulus trains produced pupillary constrictions. Others also have “often” elicited pupillary constriction by stimulation at the sites of luminance neurons (Gamlin et al. 1995). Furthermore, when we either drove the electrode 250 μm beyond the luminance neuron or withdrew it by 250 μm, pupillary constriction could not be elicited at the same stimulus strength. Therefore the effective loci for electrical stimulation were local to the site of the luminance neurons. The latency for electrical stimulation (112.4 ± 27.3 ms) was not statistically different (P > 0.05) from the average latency from the burst to the onset of pupillary constriction (94.4 ± 22.2 ms), suggesting that we were activating the light reflex loop at the level of the PON.

At three of the four stimulation sites, constriction was followed by dilation before the stimulation ended (Fig. 9). Experiments in the rat PON produced a similar result (Trejo and Cicerone 1984) (Fig. 3). The apparently premature dilation is similar to a phenomenon called “pupillary escape,” in which the pupil constricts to a low-intensity light but does not remain constricted while the stimulus is still on (Lowenstein and Loewenfeld 1959). If the same mechanism is at work in our study, a higher stimulation strength may have yielded a maintained constriction. The fact that the dilation occurred before electrical stimulation ceased suggests that the cause of pupillary escape exists in a structure downstream from the PON.

**Do transient neurons participate in the light reflex?**

The relation of the discharge of burst-sustained neurons with stimulus luminance makes them suitable candidates to be light reflex relay neurons. Since the change in pupillary area also increases linearly with the log of stimulus luminance, input signals from the PON would be perfect to facilitate that relation. In contrast, the transient neurons do not seem appropriate to participate in a light reflex that depends on luminance as they show no consistent relation to that stimulus parameter. Also the typical transient neuron illustrated in Fig. 2B discharges a burst at light-on that is associated with a pupillary constriction but a burst at light-off that is associated with dilation. Such inconsistent behavior seems inappropriate for a relay neuron of the pupillary light reflex. Instead, transient neurons are probably part of pretectal circuits with other functions.

Although transient neurons have been recorded in the pretectum of the cat, they lie superficial to the feline PON and, furthermore, cannot be antidromically activated from stimulation of the EW nucleus (Distler and Hoffmann 1989). Also there is no mention of transient neurons in the other report on neural recordings in the PON of the monkey (Gamlin et al. 1995). Therefore we suggest that transient neurons probably are not a second relay neuron in the light reflex pathway and PON burst-sustained neurons are solely responsible for the pupillary light reflex. Furthermore, PON neurons are specifically involved with pupillary constrictions associated with changes in light intensity as they do not change their activity for pupillary constriction associated with the near response (Zhang et al. 1996).

**Timing in the light reflex pathway**

Our data suggest that the latency from the stimulus to the burst (Fig. 3A) is less variable than the latency from the burst to the pupillary constriction (Fig. 3B). It is not surprising that the burst occurs at a relatively less variable latency after the stimulus because the pretectum lies only one synapse from retinal ganglion cells. On the other hand, between these putative pretectal relay neurons and pupillary constriction itself, there are synapses in the EW nucleus, the ciliary ganglion and at the pupillary muscles. Furthermore, there appears to be convergence from the contralateral PON as well (Büttner-Ennever et al. 1996; Steiger and Büttner-Ennever 1979). Bringing both the EW cells and those in the ciliary ganglion to threshold could well be a rather variable process. Therefore the multiple synapses, the requirement of convergence, and the less machine-like contractions of smooth muscle may all contribute to making the latency from the PON to pupillary constriction more variable.

If the neurons in the direct pupillary light reflex really number only three or four, why is the latency from light stimulus to pupillary response so long, i.e., ≥128 ms even with...
the brightest light in the most rapidly responding subject, monkey T (Pong and Fuchs 2000)? In the experiments in this paper, the shortest reflex latencies to the brightest stimulus were 150 (Fig. 2A) and 173 ms (Fig. 3A). In those same experiments, the latency to the burst of the burst-stimulated units averaged 51.9 ms. This suggests that the retinal ganglion cells that are involved are among the slowest, likely W cells. Indeed, in the cat, the principal pretectal input does originate from W cells (Cleland and Levick 1974; Stone and Fukuda 1974). No similar information is currently available for the monkey. The time from activation of PON neurons to the onset of pupillary constriction, whether determined by electrical stimulation (112.4 ms) or by the latency from burst onset (94.4 ms), yields comparable values, which average ~100 ms. Because it is thought that there are at most two neurons interposed between the PON and the iris muscle, it seems likely that the 100-ms delay is taken up in activating the sluggish pupillary constrictor muscle. This indeed seems to be the case because electrical stimulation of the EW nucleus or the oculomotor nerve produced pupillary constriction with latencies of 80 to 120 ms (Clarke and Gamlin 1995).

In conclusion, the pupillary light reflex transforms a sensory signal into a motor output through as few as four neurons between the retina and the pupillary sphincter muscle. The data from our study show that the sensorimotor transformation has not yet occurred at the PON, the site of the first relay neuron after the retina, because PON neural discharge rates and timing reflect the sensory stimulus more than the motor action. Therefore the sensorimotor transformation must occur at the EW nucleus, where the neural discharge would be expected to have a strong relation with the pupillary constriction parameters, a relation that is missing at the level of PON luminance neurons.

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