Primate Pupillary Light Reflex: Receptive Field Characteristics of Pretectal Luminance Neurons

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Clarke, Robert J., Hongyu Zhang, and Paul D. R. Gamlin. Primate pupillary light reflex: receptive field characteristics of pretectal luminance neurons. J Neurophysiol 89: 3168–3178, 2003. First published January 15, 2003; 10.1152/jn.01130.2002. This study examined the response properties of luminance neurons found within the pretectal olivary nucleus (PON), which is the pretectal nucleus that mediates the primate pupillary light reflex. We recorded the activity of 121 single units in alert, behaving rhesus monkeys trained to fixate a back-projected laser spot while a luminance stimulus was presented. The change in the firing rate of luminance neurons was measured as a function of changes in the size, retinal illuminance, and position of the stimulus. We found that these neurons possessed large receptive fields, which were sufficiently distinct that they could be placed into three classes. Approximately 40% of the PON luminance neurons responded well to stimuli presented in either the contralateral or ipsilateral hemifield. These neurons were classified as “bilateral” neurons. In the primate, retinal projections to the pretectum and other retinorecipient nuclei are organized such that direct retinal input can only account for the contralateral hemifield responses of these neurons. Thus the representation of the ipsilateral hemifield in “bilateral” PON cells must result from input from a nonretinal source. Approximately 30% of PON neurons responded only to stimuli presented in the contralateral hemifield. These neurons were classified as “contralateral” neurons. Finally, approximately 30% of PON neurons responded to stimuli presented at or near the animal’s fixation point. These neurons were classified as “macular” neurons. The mean firing rates of all classes of neurons increased with increases in stimulus size and luminance within their receptive fields. The thresholds and magnitude of these responses closely matched those that would be appropriate for mediating the pupillary light reflex. In summary, these results suggest that all three classes of PON neurons contribute to the behaviorally observed pupillomotor field characteristics in which stimuli at the macular produce substantially larger pupillary responses than more peripheral stimuli. The contributions of “bilateral” and “contralateral” cells account for pupil responses evoked by peripheral changes in luminance, whereas the contributions of all three cell classes account for the larger pupillary responses evoked by stimuli in the central visual field.

INTRODUCTION

The pupillary light reflex (PLR) is the constriction of the pupil that is elicited by an increase in illumination of the retina. The pretectum has been known to play an important role in the PLR since the pioneering studies of Magoun, Ranson, and colleagues in the 1930s (Magoun et al. 1935; Ranson and Magoun 1933). Their studies in cats and monkeys showed that the PLR involves synapses in the pretectum, Edinger-Westphal (EW) nucleus, and ciliary ganglion. Because the pretectum comprises seven distinct nuclei, at least five of which receive a direct retinal projection, there was some uncertainty as to the identity of the pretectal nucleus responsible for the PLR (Berman 1977; Hutchins and Weber 1985; Kanaseki and Sprague 1974; Scalia 1972; Scalia and Arango 1979; Weber and Hutchins 1982). However, it has now been shown that only the pretectal olivary nucleus (PON) has a direct projection to the EW nucleus, and it is this direct pathway that provides the anatomical substrate for the PLR (Benevento et al. 1977; Breen et al. 1983; Büttner-Ennever et al. 1996; Carpenter and Pierson 1973; Gamlin and Clarke 1995; Gamlin et al. 1984; Klooster et al. 1995a,b; Kourouyan and Horton 1997; Pierson and Carpenter 1974; Steiger and Büttner-Ennever 1979; Young and Lund 1994). Further support for a pupillomotor role for the PON has come from electrophysiological studies that have shown that neurons in this nucleus display the characteristics expected for neurons mediating the PLR (Clarke and Ikeda 1985a,b; Distler and Hoffmann 1989a; Gamlin et al. 1995; Pong and Fuchs 2000; Trejo and Cicerone 1984). Furthermore, it has been shown that electrical microstimulation of the PON elicits pupilloconstriction (Chan et al. 1995; Gamlin et al. 1995; Pong and Fuchs 2000; Trejo and Cicerone 1984).

Apart from three recent studies (Gamlin et al. 1995; Pong and Fuchs 2000; Zhang et al. 1996), all other studies of the electrophysiological bases of the PLR have been conducted in anesthetized animals such as cats and rats. However, it has become apparent that the use of alert animals is advantageous for studies of the PLR because it ensures that pupil diameter is appropriately modulated by changes in illumination and that the potentially compromising effects of anesthesia on neuronal responses are avoided. In addition, non-human primates are a very good choice for studies of the PLR because unlike those of cats and rats, the direct and consensual pupillary responses in both humans and non-human primates are equal in amplitude.

The previous studies of the PLR in alert rhesus monkeys only examined the response of PON neurons to wide-field luminance changes. These studies reported the existence of burst-sustained PON luminance neurons with firing rates that increased as a function of increases in retinal illumination (Gamlin et al. 1995; Pong and Fuchs 2000). To better understand the way in which retinal luminance signals are integrated...
in the pretectum, we further characterized PON neurons with respect to their receptive fields, luminance sensitivities, spatial summation, and ocular dominance properties.

METHODS

Preparation of animals

Five adult monkeys (Macaca mulatta; 4 male, one female, aged 3–8 yr) were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee and complied with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Surgical procedures that have been reported previously are only briefly described (Gamlin et al. 1994). Animals underwent three sterile surgical procedures under pentobarbital sodium anesthesia. Postsurgically they received analgesics to minimize pain. Animals were first implanted with a stainless steel head-holder in the first surgery. Then, to measure eye position, 4–10 wk later a search coil was implanted under the conjunctiva of one eye in the second surgery (Fuchs and Robinson 1966; Judge et al. 1980). Once animals reached a satisfactory level of training, a second eye coil was implanted on the other eye. In this final surgery, two chambers, one on each side of the head, were implanted stereotaxically over the pretectum at an 18° angle to the sagittal plane over 15-mm holes trephined in the skull. Methods for behavioral training, stimulus presentation, and eye movement recording are described in the accompanying paper (Clarke et al. 2003).

Unit recording and electrical microstimulation

Using a Kopf microdrive, a parylene-insulated tungsten microelectrode mounted in a 26-gauge cannula was advanced through a 21-gauge hypodermic syringe inserted through the dura. Unit activity was filtered sharply above 5 kHz, and the occurrence of a spike was detected with a window discriminator and recorded to disk with a resolution of 0.1 ms. As needed, the microelectrode could be switched to electrical microstimulation, consisting of biphasic stimuli of 0.2 ms total duration at a frequency of 200–500 Hz as generated by a Grass S88 stimulator and two stimulus isolation units. Microstimulation currents were generally 40 μA and never exceeded 100 μA.

Histology and verification of recording site

Animals were used for several months, and marking lesions could not be made at all relevant sites. However, records were kept of the location of familiar landmarks, the X-Y location of the micropositioner, and the depth at which cells of interest were located. To verify the location of our electrodes, marking lesions were made during the last 2 wk of recording at the site of luminance neurons by passing 30-μA anodal current through the microelectrode for 20 s. Subsequently, monkeys were anesthetized deeply with pentobarbital sodium and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were cut coronally and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were cut coronally at 2-mm thickness, and a Nissl series was prepared. The marking lesions were retrieved from these histological sections.

Data analysis

The recorded data were analyzed off-line using a computer equipped with interactive graphics. Individual trials showing eye positions, pupillary diameter, and target position, size, and intensity were displayed. Below these traces, concurrent single-unit activity was also displayed.

Based on previous studies (Gamlin et al. 1995; Pong and Fuchs 2000), we confined our analysis to burst-sustained luminance neurons that responded with sustained activity throughout the presentation of the stimuli. The spontaneous firing rate of a neuron was measured while the monkey fixated the laser spot on the tangent screen with background luminance of 10–4 cd/m2. This baseline activity was subtracted from the sustained activity (averaged over 1–4 s) elicited by changes in illumination to produce a measure of the change in firing rate that resulted from the presentation of light stimuli of varying size, intensity and position. Mesh plots of these changes in activity were generated and plotted on three-axis graphs. These plots enabled us to characterize the receptive field profiles of the neurons.

To characterize the luminance sensitivity and spatial summation characteristics of individual neurons at various locations within the visual field, the data were fitted using the formulas

\[
FR = FR_{\text{max}} \times \frac{L^b}{(L^b + \mu)}
\]

where \( FR \) is change in firing rate; \( FR_{\text{max}} \) is maximal firing rate; \( L \) is luminance (cd/m2); \( A \) is stimulus area (degrees²); \( b \) and \( \mu \) are constants. No attempt was made to fit the data unless records were available for luminance between 0.03 and 30 cd/m2 or for stimulus diameters of between 2 and 16°.

Analysis of ocular dominance

The strength of the visual input to tonic-on pretectal neurons was determined using monocular Maxwellian viewing. The change in firing rate above the spontaneous rate was measured over a 1,000-fold range of retinal illumination. The monocular data were fitted using the formula

\[
FR = FR_{\text{max}} \times \frac{L^b}{(L^b + \mu)}
\]

where \( FR \) is change in firing rate; \( FR_{\text{max}} \) is maximal firing rate; \( L \) is luminance (Trolands); \( b \) and \( \mu \) are constants. The firing rate at 500 Trolands was derived from the best fit curve for each eye and the ocular dominance calculated from the equation

\[
OD = \frac{FR(C) - FR(I)}{FR(C) + FR(I)}
\]

Where \( OD \) = ocular dominance ratio; \( FR(C) \) = change in firing rate with contralateral eye stimulation; \( FR(I) \) = change in firing rate with ipsilateral eye stimulation.

RESULTS

Receptive fields of pretectal luminance neurons

The responses of 121 pretectal ON luminance neurons were investigated. All neurons showed characteristic burst-sustained activity in response to the appearance of a visual stimulus within their receptive fields. Specifically, they responded with an initial transient followed by a sustained increase in firing rate that lasted for the duration of the stimulus presentation. The recording sites for these neurons were restricted to a small area approximately 0.25 mm in anterioposterior extent and 0.75 mm in mediolateral extent. The location and dimensions of this area corresponds to the PON, a nucleus that has previously been demonstrated to contain ON luminance neurons with properties suitable for mediating the PLR (Gamlin et al. 1995; Pong and Fuchs 2000). The receptive fields of 84 of these luminance neurons were determined using a visual stimulus to probe much of the visual field. The responses of the remaining 37 neurons were systematically characterized along the horizontal meridian. Within the region that appeared to correspond to the PON, we encountered no accommodation-related or eye movement-related neurons. Posterior to the
example of each of these three types of receptive fields is shown in Fig. 1. Figure 1A shows a receptive field with a flat response profile, indicating that the neuron responded equally well to the stimulus irrespective of where it was presented in the visual field. Neurons with this receptive field profile are referred to as “bilateral” cells. Figure 1B shows the behavior of a class a neuron that gave maximal responses for stimuli presented less than 10° from the fixation point. The response of these neurons was much reduced when the stimulus was presented in the periphery. Because this class of neurons responded preferentially to stimuli presented within the region of the visual field viewed by the macula, these neurons are referred to as “macular” neurons. Figure 1C depicts the receptive field of a neuron that responded best to stimuli placed in the visual field contralateral to the recording side. These neurons are referred to here as “contralateral” neurons. Neurons belonging to each of these receptive field classes were encountered throughout the recording area, with no evidence of clustering or topography.

“Bilateral” neurons

We found that bilateral neurons formed the largest group of neurons (47/121 cells), comprising 40% of the total population of classified cells. Figure 2 (A–E) shows histograms of the responses of one such neuron to a 5° stimulus placed at specific locations along the horizontal meridian. For each stimulus location, the neuron responded after a short delay with a burst-sustained firing pattern in response to the appearance of the stimulus. The neuron’s sustained discharge remains elevated for the duration of the trial until the stimulus is extinguished. It is clear from this figure that the visually evoked changes in firing rates are of approximately the same magnitude irrespective of whether the stimulus was positioned in the contralateral (Fig. 2, A and B) or ipsilateral visual field (Fig. 2, D and E) or at the fovea (Fig. 2C). This is also seen in Fig. 3 where data for the population of bilateral cells is plotted. The thick line represents the average response of the population at each eccentricity. In general, a slightly greater response is elicited from the contralateral visual field than the ipsilateral field, as shown by the slightly lower firing rate with ipsilateral field presentations.

To analyze further the bilateral receptive fields of these neurons, their responses to stimuli placed 30° eccentrically either in the contralateral or ipsilateral hemifield were charac-
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FIG. 3. Summary plot of the behavior of 31 pretectal luminance neurons that showed bilateral responses to stimuli presented along the horizontal meridian. The change in firing rate that resulted from stimulus presentation is shown as a function of stimulus location. The thick line represents the mean response at each position along the horizontal meridian. Contralateral visual field is to the left in the figure.

Curves were fitted through the steady-state firing rates of these neurons for stimuli that were varied systematically over a wide range of luminance (Fig. 4, A and B) and size (C and D) in the contralateral (A and C) and ipsilateral (B and D) hemifield. Based on the systematic and monotonic increases in neural activity seen with increases in stimulus area (C and D), it is clear that “bilateral” neurons display substantial spatial summation within their receptive fields. Further quantitative analysis was performed for the data shown in A and B. This analysis indicated that the intensity of illumination required to produce half-maximal firing rates varied greatly between neurons but was not significantly different for contralateral and ipsilateral stimuli. Measured values ranged between 0.6 and 180 cd/m² (mean = 5.0 cd/m²) for contralateral stimuli and 0.5 and 125 cd/m² (mean = 5.7 cd/m²) for ipsilateral stimuli. This analysis also indicated that the mean maximal firing rates were not significantly different between the contralateral and ipsilateral hemifields (27 vs. 25 spikes/s respectively).

Macular neurons

We found that macular neurons (36/121 cells) comprised approximately 30% of the total population of classified cells. Figure 5 (A–E) shows the response of a macular neuron to a 5° stimuli placed at various locations along the horizontal meridian. This neuron responded only to stimuli presented near the fixation point (Fig. 5C). For this stimulus location, after a short delay, the neuron responded in a burst-sustained fashion in response to the appearance of the stimulus. The neuron’s sustained discharge remained elevated for the duration of the trial until the stimulus was extinguished. It is clear from this figure that the neuron responded poorly to stimuli presented in the contralateral (Fig. 5, A and B) or ipsilateral visual field (D and E). This is also seen in Fig. 6 where data for the population of macular cells is plotted. The thick line represents the average response of the population at each eccentricity. A clear peak of activity can be seen for foveally presented stimuli. Responses are much reduced for stimuli in the periphery, especially within the ipsilateral hemifield. To more extensively analyze the characteristics of these neurons, we examined their responses to stimuli that were presented at the fixation point and systematically varied over a wide range of luminance (Fig. 7A) and size (B). We found that the mean maximal firing rate for stimuli in the macula was significantly higher (34.6 spikes/s, P < 5%) than that seen for stimuli presented either contralaterally or ipsilaterally. Our analyses also showed that while the mean maximal firing rate of macular neurons was not significantly higher than that of bilateral or contralateral neurons in response to stimuli in the contralateral visual field, it was significantly (P < 1%) higher in response to stimuli in the ipsilateral visual field. This analysis also indicated that the intensity of illumination required to produce half-maximal firing rates varied greatly between neurons (range 0.2 and 60 cd/m²), but that the mean value (3.8 cd/m²) was not significantly different to that seen for bilateral or contralateral neurons for stimuli within their receptive fields. Based on the systematic and monotonic increases in neural activity seen with increases in stimulus area (Fig. 7B), it is clear that macular neurons display substantial spatial summation within their receptive fields. Such spatial summation was seen for stimuli of ±20° in diameter, and no reduction in activity was seen that would be indicative of an inhibitory surround.

Contralateral neurons

We found that contralateral neurons (38/121 cells) comprised approximately 30% of the total population of classified cells. Figure 8 (A–E) shows the response of a contralateral neuron to a 5° stimuli placed at various locations along the horizontal meridian. This neuron responded only to stimuli presented in the contralateral hemifield (Fig. 8, A and B). It is clear from Fig. 8 that the neuron responded poorly to stimuli at the fixation point (Fig. 8C) and in the ipsilateral hemifield (Fig. 8, D and E). This is also seen in Fig. 9 in which the data for the population of contralateral cells is plotted. The thick line
represents the average response of the population at each eccentricity. Clearly, these neurons respond significantly to stimuli presented in the contralateral visual field but not to stimuli presented in the ipsilateral visual field. To analyze the receptive fields of these neurons, their responses to stimuli placed 30° eccentrically either in the contralateral or ipsilateral hemifield were characterized. Curves were fitted through the mean firing rates of these neurons for stimuli that were varied systematically over a wide range of luminance (Fig. 10, A and B) and size (Fig. 10, C and D) in the contralateral (A and C) and ipsilateral (B and D) hemifield. Based on the systematic and monotonic increases in neural activity seen with increases in stimulus area (Fig. 10C) for stimuli in the contralateral visual field, it is clear that contralateral neurons display substantial spatial summation within their receptive fields. Further quantitative analysis was performed for the data shown in Fig. 10, A and B. It was found that the mean maximal firing rate (27 spikes/s) of contralateral neurons was not significantly different from that of bilateral or macular neurons for stimuli within their receptive fields. This analysis indicated that the intensity of illumination required to produce half-maximal firing rates in macular neurons but are significantly (P < 5%) lower than those required to produce half-maximal firing rates in bilateral neurons. For stimuli in the ipsilateral visual field, it was generally only possible to elicit substantial responses with bright (more than 100 cd/m²) or large stimuli (100°²). At these high light levels, it is unclear if the observed responses are the result of a weak ipsilateral responsiveness, or of light straying into the neuron’s receptive field in the contralateral hemifield.

FIG. 5. Activity of a typical macular receptive field pretectal luminance neuron in response to a 35 cd/m², 5° stimulus presented along the horizontal meridian. Stimuli were located 40° (A) and 20° (B) in the visual field contralateral to the recording side, 20° (D) and 40° (E) in the visual field ipsilateral to the recording side, and at the fixation point 0° (C). Stimulus onset occurred at 1 s.

FIG. 6. Summary plot of the behavior of 22 pretectal luminance neurons that showed macular receptive field responses to stimuli presented along the horizontal meridian. The change in firing rate that resulted from stimulus presentation is shown as a function of stimulus location. The thick line represents the mean response at each position along the horizontal meridian. Contralateral visual field is to the left in the figure.

FIG. 7. Graphs showing luminance sensitivity and spatial summation characteristics of macular receptive field neurons. Each curve was fitted through the mean firing rates of a cell. Stimuli were presented at the fixation point. A is a plot of responses to changes in luminance of a 5° stimulus. B: plot of responses to changes in size of a 35 cd/m² stimulus.
Ocular dominance of PON neurons

We used a binocular Maxwellian viewing system (Clarke et al. 2003) to examine the relative input from each eye to PON luminance neurons. Using this system, we could examine the responses of PON neurons to illumination of each eye independently. We found that the responses of most neurons were driven by inputs from both eyes, although some neurons were driven exclusively by the contralateral eye. The results obtained from 25 PON neurons are shown in Fig. 11. Overall, we found that the number of cells driven equally by both eyes accounted for 44% of the total. No cells were dominated by the eye ipsilateral to the recording side. Of the remaining neurons, we found that the responses of 56% were dominated by the eye contralateral to the recording side with 16% being driven exclusively by that eye.

DISCUSSION

We have investigated the properties of luminance neurons in the primate PON. These pretectal neurons are the central interneurons between the pupillomotor retinal input and preganglionic output to the ciliary ganglion and presumably mediate the PLR. Previous studies had reported the existence of burst-sustained PON luminance neurons with firing rates that increased as a function of increases in retinal illumination (Gamlin et al. 1995; Pong and Fuchs 2000). We have confirmed these findings and found that the population thresholds and magnitude of the neuronal responses are appropriate for mediating the PLR. There is clear evidence that three classes of PON neurons exist with distinctly different receptive field organizations and, surprisingly for a retinorecipient nucleus in the primate, the most numerous of these classes of PON neurons possess bilateral receptive fields. In addition, examination of pretectal ocular dominance characteristics revealed that all PON luminance neurons either are dominated by input from the contralateral eye or have balanced ocular dominance characteristics.

Receptive field properties of PON luminance neurons

PON luminance neurons of the bilateral and contralateral classes possess very large receptive fields, which exceed
We estimated that their receptive fields described the receptive fields (RGCs) over a wide area of retina. In a recent report, we described the receptive fields of the luminescent RGCs that project to the pretectum and presumably mediate the PLR (Gamlin et al. 2001). We estimated that their receptive field areas averaged approximately $7^\circ$ centrally and $20^\circ$ peripherally. Thus it would require input from hundreds of luminescent RGCs to generate the receptive fields possessed by bilateral and contralateral neurons. In contrast, the receptive fields (approximately $100-900^\circ$) of macular PON neurons could be constructed with input from approximately 50 (range 20–100) centrally located luminescent RGCs.

The most important result of this study was the finding that many PON neurons receive input from retinal ganglion cells (RGCs) over a wide area of retina. In a recent report, we described the receptive fields of the luminescent RGCs that project to the pretectum and presumably mediate the PLR (Gamlin et al. 2001). We estimated that their receptive field areas averaged approximately $7^\circ$ centrally and $20^\circ$ peripherally. Thus it would require input from hundreds of luminescent RGCs to generate the receptive fields possessed by bilateral and contralateral neurons. In contrast, the receptive fields (approximately $100-900^\circ$) of macular PON neurons could be constructed with input from approximately 50 (range 20–100) centrally located luminescent RGCs.

The most important result of this study was the finding that many PON neurons possess extensive bilateral receptive fields; this is not consistent with the traditional concept of the visual field representation in primate retinorecipient nuclei. In the primate, apart from a small amount of nasotemporal overlap, ganglion cells in the nasal retina project to the PON on the contralateral side of the brain, and ganglion cells in the temporal retina project to the ipsilateral PON (Kondo et al. 1992; Perry and Cowey 1984; Rodieck and Watanabe 1993). Thus one would expect PON neurons to possess an entirely contralateral visual field representation, just as is the case for most other retinorecipient nuclei. It might be suggested that the responses of these neurons to stimuli in the ipsilateral visual field result from light scatter. However, the response characteristics of the contralateral neurons demonstrate that this cannot be the case. Presumably, light scatter would affect all classes of neurons equally, but although the bilateral PON luminance neurons display robust responses to stimuli presented in the ipsilateral visual field, the contralateral neurons show no responses to such presentations except for very bright or large stimuli.

We believe that the ipsilateral visual field responses of PON luminance neurons must result from nonretinal inputs. One obvious source is the contralateral PON. However, recent anatomical studies in macaques have failed to demonstrate any such reciprocal connections between the pretectal olivary nuclei (Baleydier et al. 1990; Mustari et al. 1994). The cerebral cortex is another possible source of the ipsilateral visual field input to the PON. In humans, lesions of the visual cortex can produce pupillary hypokinesia (Barbur and Forsyth 1986), and psychophysical studies suggest cortical inputs to the PLR (Barbur and Forsyth 1986; Young and Kennish 1993). In cats, an anatomical and electrophysiological study showed that areas 19 and 20a project to the olivary pretectal nucleus (Distler and Hoffmann 1989b). In monkeys, anatomical studies have repeatedly identified well-defined projections from both the dorsal and more ventral regions of the prelunate gyrus to the ipsilateral PON in monkeys (Asanuma et al. 1985; Dineen and Hendrickson 1983; Leichnetz 1990; Lui et al. 1995). Furthermore, there is clear evidence for cortical involvement in the generation of the ipsilateral component of the receptive fields of another pretectal nucleus, the nucleus of the optic tract (NOT). The NOT is located immediately dorsolateral to the PON, and its neurons possess receptive fields that extend ipsilaterally approximately $20^\circ$ beyond the vertical meridian (Hoffmann et al. 1992). Section of the corpus callosum abolishes this ipsilateral hemifield representation (Hoffmann et al. 1992). A comparable pathway involving the corpus callosum may exist that gives rise to the ipsilateral visual field representation in bilateral PON luminance neurons.

**Correlation between neuronal receptive fields and pupillomotor receptive fields**

One of the objectives of this study was to correlate the activity of PON luminance neurons with that of the PLR to more fully evaluate their participation in the PLR. Consistent with such participation, we found that the thresholds and sensitivities of PON luminance neurons closely matched those of the pupillary responses. For example, using a $5^\circ$ stimulus placed $30^\circ$ from the fovea, the intensity of illumination required to produce half-maximal firing rates for bilateral neurons was approximately 5 cd/m$^2$, whereas that required to produce half-maximal pupil constriction was 10 cd/m$^2$ (Clarke et al. 2003) (Fig. 10). Furthermore, we found that the firing rates of PON neurons very rarely exceed 50 spikes/s and thus closely match the activity of neurons in their target, the EW nucleus. There the preganglionic neurons that mediate accommodation rarely fire more than 40 spikes/s (Gamlin et al. 1994), and the activity of one recorded preganglionic pupillomotor neuron in the EW was similar (Gamlin 2000). Further, these firing rates correspond to the physiological range of frequencies over which the smooth muscle of the iris sphincter operates (Gamlin and Clarke 1996).

Another objective of this study was to correlate PON luminance neuron receptive field profiles with specific features of the pupillomotor response fields (Clarke et al. 2002). We found that none of the individual receptive fields of PON neurons matched the overall pupillomotor response fields. Thus it is clear that no single receptive field class of PON luminance neurons is sufficient to explain the observed pupillomotor response field characteristics. The profile of the pupillomotor response fields can be best, and most parsimoniously, accounted for by a combination of all three PON receptive fields. This is not surprising as the pretectum is not the final common pathway of the PLR. Therefore it seems likely that neurons belonging to all three receptive field type classes project to the EW nucleus. The receptive fields of EW neurons would thus reflect...
the combination of macular, contralateral, and bilateral receptive field inputs, which together resemble the profile of the pupillomotor response field as shown in Fig. 12. Therefore we propose that the integration of the PON neuronal receptive field properties that are required to produce a pupillomotor response field takes place in the EW nucleus through the direct projection of PON to the EW nucleus. Under normal circumstances, signals from neurons in the left and right PON combine directly at the EW nucleus, therefore a signal such as shown in Fig. 12 for the unilateral PON output stage would not be observed. However, we predict that unilateral inactivation of the PON would reveal such a signal.

“Tonic-off” or darkness-detecting neurons have been reported in the pretectum (Clarke and Ikeda 1985a), and oculomotor complex (Smith et al. 1970). These cells have higher tonic firing rates in the dark and lower rates at high luminances. It has been postulated that these cells function as inhibitory interneurons working in opposition to tonic-on neurons in the parasympathetic pathway. These two functional cell types may function in a “push-pull” arrangement to control pupil size, i.e., tonic-off neurons dilate the pupil in the dark while tonic-on cells constrict the pupil in bright light (Clarke and Ikeda 1985a; Sillito and Zbrozyna 1970). This hypothesis has been questioned for a number of reasons. First these cells occur in very small numbers within the posterior pretectal nucleus in the rat (Clarke and Ikeda 1985). Similar cells were encountered in this study in the monkey in a location caudal to the PON that corresponded roughly to the rostral superior colliculus or cau-

**Fig. 12.** This schematic diagram shows bilateral PON stages each with 3 neurons possessing the receptive fields characteristics of the 3 classes of PON neurons. Output from the PON is combined bilaterally at the Edinger-Westphal nucleus (EW) to produce the output of this nucleus. This output then produces the observed pupillary responses as shown in the bottom (from Clarke et al. 2003, Fig. 8B). In this schematic diagram, before the PON signals converge on the EW nucleus, a summing junction is shown as combining unilaterally the signals from each class of PON neuron as a weighted sum based on their prevalence (bilateral = 0.4; macular = 0.3; contralateral = 0.3). However, because the PON has direct, bilateral projections to the EW nucleus, the response shown at the summing junction would not normally be seen and is presented only as a diagrammatic convenience.
Second, there is no direct evidence for a well-defined anatomical substrate for such an inhibitory pathway. Retrograde tracer studies show that only the PON and NPC, which do not contain tonic-off neurons, project to the oculomotor complex (Ben-evento et al. 1977; Büttner-Ennever et al. 1996; Carpenter and Pierson 1973; Carpenter et al. 1992; Gamlin and Clarke 1995; Pierson and Carpenter 1974; Steiger and Büttner-Ennever 1979).

### Binocularity of pretectal luminance neurons

The ocular dominance histogram in Fig. 11 shows that all PON luminance neurons receive input from the contralateral eye. In addition, it shows that PON luminance neurons often receive input from the ipsilateral eye. However, the input from the ipsilateral eye never dominates that from the contralateral eye and is absent in some cases. These observations are entirely consistent with the results of anatomical studies. Intravitreal injections of anterograde tracers have shown heavy projections from both eyes to the monkey PON with the density of retinal afferent terminals being greatest in the PON contralateral to the injected eye (Gamlin and Clarke 1995; Hutchins and Weber 1985; Weber et al. 1981). Furthermore, Hutchins and Weber (1985) described a laminated organization of the retinal terminal fields within the monkey PON suggesting that ocular inputs might be segregated within the nucleus and that significant numbers of PON neurons might display monocular responses, but we encountered only a few (16%) monocular cells.

### Comparison to previous studies

Previous studies have investigated the responses of pretectal pupillomotor neurons in rats, cats, and macaque monkeys. Only two studies, one in rat (Trejo and Cicerone 1984) and the other in cat (Distler and Hoffmann 1989), have previously investigated the receptive field characteristics of PON neurons. A study in rat reported that tonic-on cells in the PON possessed receptive fields of mean diameter 31° with large, weak inhibitory surrounds. It was suggested that such receptive fields could be formed by the convergence of inputs from approximately 10 tonic-on retinal ganglion cells (Trejo and Cicerone 1984). The study in cat reported tonic-on cells with receptive fields of between 10 and 40° with indistinct borders that were all located contralaterally. Thus no previous study has reported PON neurons with such extensive receptive fields as observed for the bilateral and contralateral neurons in the primate PON. In addition, while 84% of PON neurons are binocular in the primate, only 22% are reported to be binocular in cats (Distler and Hoffmann 1989). Thus it is likely that PON neurons with such extensive, binocular receptive fields are unique to primates, and the existence of such neurons may explain why the magnitude of the direct and consensual pupillary responses are comparable in primates. The apparent absence of such PON neurons in rodents and cats may explain why the direct pupillary responses in these species are substantially larger than the consensual responses.

In all species studied, the threshold for a sustained response of the luminance cells is reported to be approximately 0.1 cd/m². Also the peak sustained firing rates of PON neurons are low in all reported cases, rarely exceeding 60 spikes/s. In a previous report (Gamlin et al. 1995), our results indicated that the relationship between firing rate and light intensity was 7.5
spikes/s per log unit intensity change, while Pong and Fuchs
(2000) reported a value of 11 spikes/s per log unit intensity
change. In the present study, we used a broad range of light
intensities and found that the neural response characteristics of
the PON neurons were better fit by the Naka-Rushton equation
than by a linear relationship between light intensity and firing
rate. Nevertheless we found that the mean maximal firing rates
for all classes of PON neurons ranged from 25 to 35 spikes/s,
and that their dynamic range was approximately 3 log units of
light intensity. These values are thus consistent with those of
previous studies.

Midbrain pupilloconstrictor pathways

To produce equal direct and consensual pupillary reponses, as
seen in non-human primates and humans, the outputs from
each eye must combine equally at some point along the PLR
pathway. Despite the large population of binocularly driven
PON neurons, the responses of most cells are dominated by the
contralateral eye (Fig. 11). Thus the process of equalizing the
output from both eyes is clearly incomplete at the level of the
pretectum. Presumably, further convergence of PON efferents
at the EW nucleus is necessary to produce equal pupillary
responses in both eyes. Anatomical experiments have not
clearly indicated the details of the projection of PON to EW.
Injections of retrograde tracers into the descending pupillocon-
strictor fibers dorsal to the EW nucleus only result in labeling
of the PON contralateral to the injected side (Büttner-Ennever
However, using a transsynaptic anterograde technique, Kour-
ouyan and Horton (1997) have reported a predominantly ipsi-
lateral projection from the PON to neurons within the presumpt-
ive lateral visceral cell column of the EW nucleus. Although
these neurons are not preganglionic, they could project to
preganglionic neurons and play an as yet undetermined role in
the PLR. If the PON projects predominantly to the contralateral
EW as is suggested by retrograde studies, then, based on the
ocular dominance characteristics of the PON neurons, the
direct response would be greater than the consensual response.
This is not the case, and the direct and consensual responses
are matched in primates. Therefore it is likely that a significant
number of fibers from the PON reach the contralateral EW
either because a decussation ventral to the aqueduct or because
the dendrites of EW neurons ramify bilaterally. Alternatively,
the ipsilateral projection reported by Kourouyan and Horton
(1997) may ensure matching of the direct and consensual
responses. Figure 13 shows a schematic diagram of the PLR
pathway illustrating that in each PON, the receptive fields of
many cells include the entire visual field. It also presents
potential PON-EW projections based on a predominantly con-
tralateral projection through the posterior commissure and a
second decussation ventral to the aqueduct. This diagram fur-
ther emphasizes that due to the nonretinal ipsilateral visual
field input, there is substantial elaboration of the complete
visual field representation within each PON. This contrasts
with the current view that there is only a contralateral visual
field representation in the PON at the level of the pretectum
and that the elaboration of a complete visual field representa-
tion within the EW nucleus requires bilateral inputs from the
PON. The substantial elaboration of the complete visual field
representation within each PON, which occurs at an earlier

stage of visual processing in the PLR pathway than was pre-
viously considered, may significantly contribute to the bal-
anced temporal/nasal and direct/consensual responses that are
seen in the primate PLR.

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