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RESEARCH NOTE

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Luminance neurons in the pretectal olivary nucleus mediate the pupillary light reflex in the rhesus monkey

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Abstract In humans and other primates, an increase in luminance in either eye elicits bilateral pupilloconstriction that is essentially equal in both eyes. Current models of the neural substrate for this clinically important light reflex propose that a retinorecipient pretectal nucleus projects bilaterally to the Edinger-Westphal nucleus (EW), which contains the parasympathetic, preganglionic neurons controlling pupilloconstriction. Based on single-unit recording studies in anesthetized cats and rats, it has been further suggested that luminance neurons in only one pretectal nucleus, the pretectal olivary nucleus, mediate this reflex. However, to our knowledge, there have been no comparable electrophysiological studies in primates of the pupillary light reflex or the pretectal luminance neurons that mediate this reflex. To address this issue, single-unit recording and electrical microstimulation studies were carried out in the pretectum of alert, trained, rhesus monkeys. These studies demonstrated that the primate pretectum contains luminance neurons with the characteristics appropriate for mediating the pupillary light reflex and that these neurons are located in one retinorecipient pretectal nucleus, the pretectal olivary nucleus. Electrical microstimulation at the site of these neurons often elicited pupilloconstriction. Our results provide clear evidence for the involvement of the pretectum, and more specifically the pretectal olivary nucleus, in mediating the pupillary light reflex in primates.

Key words Pretectum · Edinger-Westphal nucleus · Pupil · Electrophysiology · Microstimulation

Introduction

The pupillary light reflex (PLR) is the constriction of the sphincter pupillae muscles of the iris that is produced by an increase in retinal illuminance. A number of anatomical, lesion, and electrophysiological studies have suggested that this reflex is mediated by projections from a retinorecipient pretectal nucleus to the Edinger-Westphal nucleus (EW), which contains the parasympathetic, pupilloconstrictor neurons of the oculomotor complex (see Loewenfeld 1993 for a review). However, in the primate, no single retinorecipient pretectal nucleus has been identified unequivocally as the source of the projection to the EW that is essential for this reflex (Pierson and Carpenter 1974; Benevento et al. 1977; Steiger and Büttner-Ennever 1979). Single-unit recording studies in anesthetized cats (Distler and Hoffmann 1989) and rats (Trejo and Cicerone 1984; Clarke and Ikeda 1985) have suggested that the PLR is mediated by luminance neurons in the pretectal olivary nucleus (PON), but, to our knowledge, there have been no equivalent electrophysiological studies of pretectal luminance neurons in the primate. Therefore, we have surveyed the pretectal complex of the alert, rhesus monkey to identify luminance neurons that could mediate the primate PLR. This report demonstrates that luminance neurons with the characteristics appropriate for mediating this reflex are located within the PON and that stimulation at the site of these neurons elicits pupilloconstriction.

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Materials and methods

Experiments were conducted in three alert, trained rhesus monkeys with general procedures that have been described previously (Gamlin et al. 1989). All experimental procedures were approved by the IACUC and complied with the USPHS Policy on Humane Care and Use of Laboratory Animals. Alert, behaving monkeys were trained to fixate a small, back-projected laser stimulus for a juice reward. They fixated this stimulus on a tangent screen at a distance of 45 cm while a second stimulus was presented binocularly in Maxwellian view through an optical system. The dimensions of the field were ±18° both horizontally and vertically. Maxwellian viewing ensured that the pupil was operating under open-loop conditions. The accuracy of fixation of both eyes was verified using the scleral search-coil technique (Fuchs and Robinson 1966). Single units were recorded extracellularly in the pretectum using platinum-plated tungsten microelectrodes lowered through

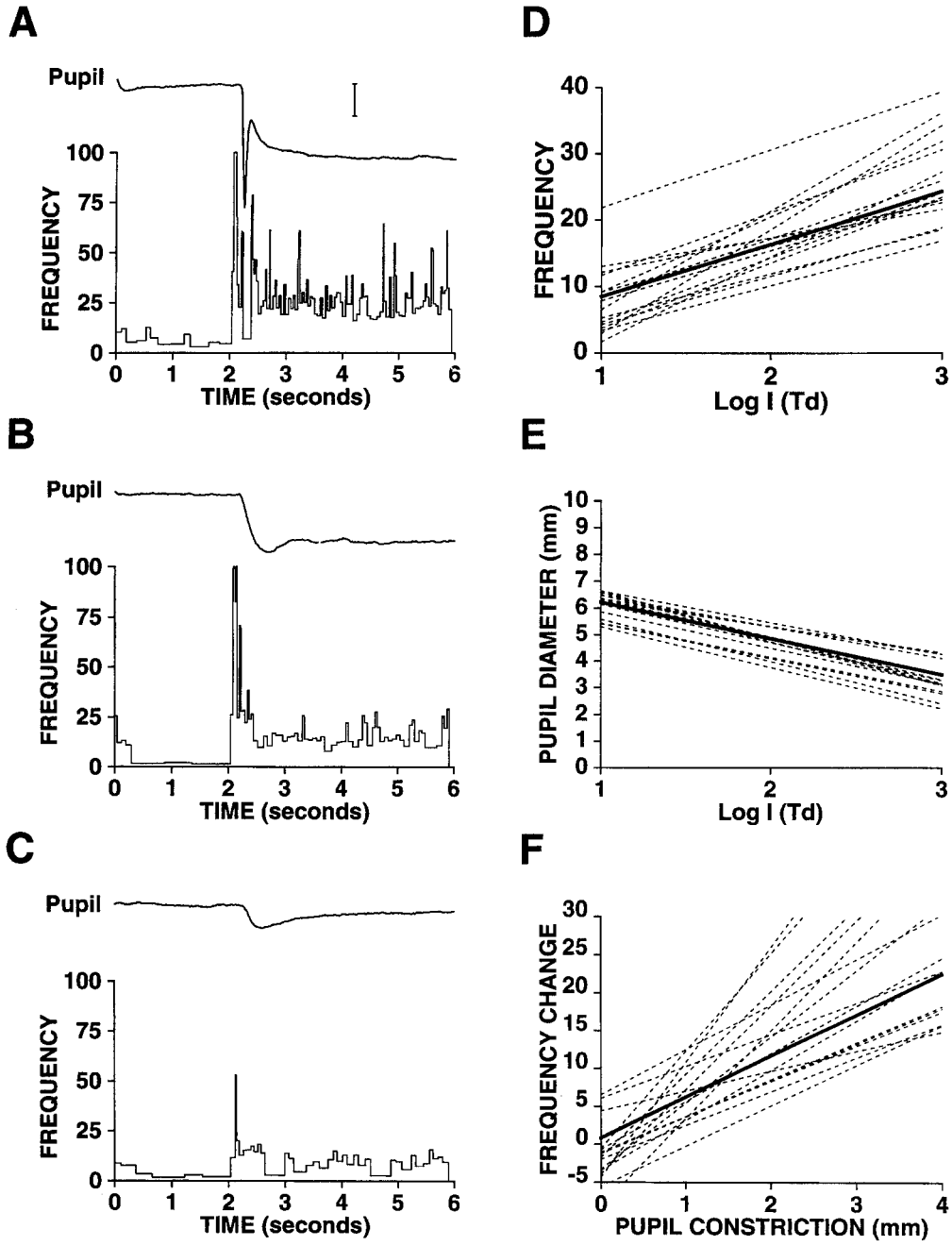

Fig. 1 A–C The response of a pretectal luminance neuron to stimuli of 1000 trolands, 100 trolands, and 10 trolands, respectively. Note that, in **A**, a blink occurs during the dynamic phase of pupilloconstriction. **D** The change in neuronal firing rate for 16 individual pretectal luminance neurons is plotted against the logarithm of retinal illuminance. The responses of the neurons were essentially linear over this range and linear regression analyses yielded correlation coefficients ranging from r=0.81 to r=0.99, with a mean correlation coefficient of r=0.92. The *solid line* in **D** is a regression line fitted to the data from all 16 neurons. In **E**, pupil diameter is plotted against the logarithm of retinal illuminance. The data were obtained while recording the luminance responses of each of the 16 cells shown in **D**. The *solid line* in **E** is a regression line fitted to the pooled data. For the 16 luminance neurons, **F** shows the mean change in neuronal firing rate as a function of pupilloconstriction. The response of the neurons was essentially linear over this range, and linear regression analyses yielded correlation coefficients ranging from r=0.8 to r=0.98, with a mean correlation coefficient of r=0.9. The *solid line* in **F** is a regression line fitted to the data from all 16 neurons. *Scale bar* 1-mm pupilloconstriction

one of two chambers implanted over 15-mm holes trephined in the skull. The two chambers, one on each side of the skull, were positioned stereotaxically over the midbrain at an 18° angle to the sagittal plane. Pupil diameters of both eyes were measured with ISCAN RK406 pupillometers. Target parameters, eye positions, pupil diameters, and the time of occurrence of action potentials were recorded to computer disk for subsequent off-line analysis.

Histology

Since each animal was used for several months, it was not possible to make marking lesions at all relevant sites. However, the location of familiar landmarks such as the superior colliculus, the *x-y* location of our micropositioner, and the electrode depth for cells of interest were noted. To verify the location of our stimulating and recording electrodes, marking lesions were made during the last 2 weeks of recording by passing 20- to 30-μA anodal current for 20 s. Animals were deeply anesthetized with pentobarbital and then perfused through the aorta with saline, followed by a suitable fixative. The brain was sectioned at 40 μm and a Nissl-stained series was prepared. The marking lesions were recovered and recording sites reconstructed using established histological procedures (Gamlin et al. 1994).

Results

Data from sixteen pretectal luminance neurons were recorded with pupillary responses placed in an open-loop condition by Maxwellian view. The responses of these neurons were sufficiently unique that they could be easily distinguished from other pretectal neurons and neu-

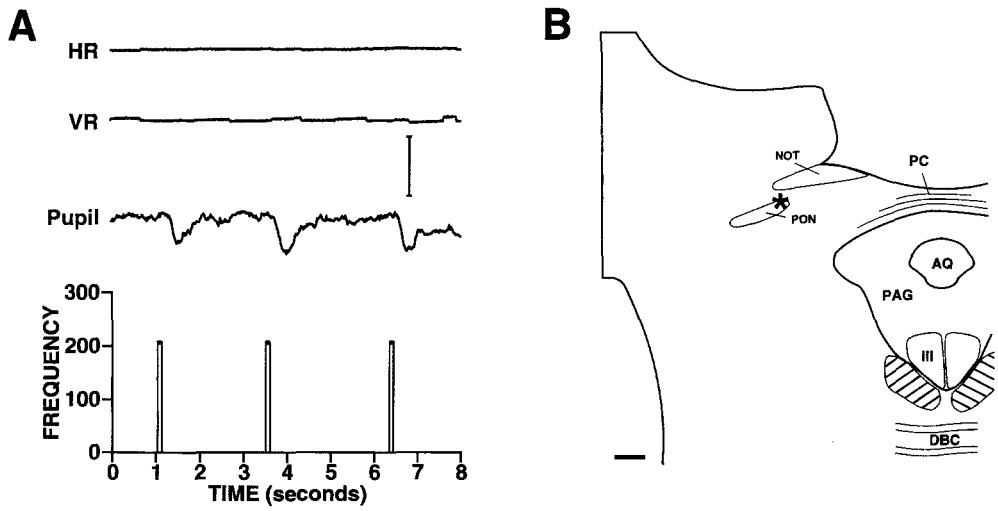

Fig. 2 **A** The effect of electrical microstimulation at the site of a pretectal luminance neuron. This shows three stimulation events (100 ms; 200 Hz; 60 μA) producing well-defined pupillary responses (*HR* horizontal right eye position, *VR* vertical right eye position). *Scale bar* 0.5-mm pupilloconstriction. **B** A line drawing of a coronal section, at the level of the pretectal olivary nucleus (*PON*), of a marking lesion (*asterisk*) placed at the location of a pretectal luminance neuron (*AQ* aqueduct, *DBC* decussation of the brachium conjunctivum, *III* oculomotor nucleus, *NOT* nucleus of the optic tract, *PAG* periaqueductal grey, *PC* posterior commissure). *Scale bar* 1 mm

rons in the superior colliculus. In response to increases in retinal illuminance, these neurons displayed a brief transient response followed by a sustained tonic response that was proportional to illuminance over the tested range of 10–1000 trolands (Fig. 1A-C). However, unlike many neurons in these areas, these neurons were not active for saccadic (Schiller and Koerner 1971; Goldberg and Wurtz 1972) or optokinetic (Mustari and Fuchs 1990) eye movements.

Figure 1D presents summary plots of the luminance sensitivity of the recorded pretectal luminance neurons. Figure 1E presents summary plots depicting the response of the pupil over the same illuminance range as in Fig. 1D. To show that the sensitivity of the pretectal luminance neurons closely matches the observed pupilloconstriction, Fig. 1F presents a plot of the change in firing rate of the pretectal luminance neurons as a function of pupil constriction. It is important to note that while the activity of some pretectal luminance cells is somewhat increased above background when pupilloconstriction is first detectable, the activity of many others is not. This is consistent with the population response of the pretectal luminance neurons (Fig. 1F, solid line), which indicates that a slight change in firing rate is seen before any pupilloconstriction can be measured. However, once the threshold for pupilloconstriction is exceeded, there is a linear relationship between increases in firing rate and pupilloconstriction over the range of tested retinal illuminance.

Electrical microstimulation at the sites of the pretectal luminance neurons often elicited pupilloconstriction. An example of one of these instances is shown in Fig. 2A. Caudal to these pretectal luminance neurons, we encountered a few scattered neurons that decreased their firing with increases in retinal illuminance. However, the activity of many of these neurons was also modulated by fixation and saccadic eye movements and they did not, therefore, seem to be directly related to the pupillary light reflex.

Location of luminance neurons

Luminance neurons were encountered within a localized region of the primate pretectum. They were confined to a region of approximately 1000 μm mediolateral, less than 500 μm anteroposterior, and approximately 300 μm dorsoventral. This is approximately the size of the PON in the rhesus monkey, and marking lesions did indeed localize luminance neurons to this nucleus. Figure 2B shows that a small lesion placed at the recording site of a pretectal luminance neuron is located in the PON. We did not encounter luminance neurons ventral to the PON in the nonretinorecipient pretectal region that corresponds to the nucleus of the posterior commissure (NPC).

Discussion

The results from these single-unit recording studies in the pretectum of alert rhesus monkeys are clear. They have demonstrated that luminance neurons with the tonic characteristics appropriate for mediating the PLR are localized to the PON. In addition, the transient responses of these neurons are also appropriate for mediating the PLR, as they would result in the preemphasis in iris innervation that is needed to overcome the low-pass characteristics of the pupil plant (Terdiman et al. 1969). Consistent with the finding that luminance neurons are localized to the PON, some anatomical studies also have reported that the PON is the only retinorecipient pretectal nucleus to project to the EW (Steiger and Büttner-En-

never 1979). Further study will be needed to better characterize the luminance neurons in the primate PON in terms of their binocularity and their spatial and temporal characteristics.

In this study, we did not encounter luminance cells ventromedial to the PON in the region that would correspond to the NPC. This is important, as it has been suggested that the pupillary light reflex pathway involves an additional synapse in the NPC, which projects to the EW (e.g., Loewy 1979). However, more recent studies have reported near-response cells in the region of the NPC (Judge and Cumming 1986; Mays et al. 1986), and it is therefore possible that the projection from the NPC to the EW is related to accommodation and not to the pupillary light reflex.

Some previous studies have suggested that the pupillary light reflex operates in a push-pull fashion, with some pretectal neurons increasing their activity with increases in luminance and other neurons decreasing their activity with increases in luminance, so-called darkness neurons (Clarke and Ikeda 1985). However, in the present study, no neurons were encountered with the characteristics of darkness detectors that were not also modulated by other oculomotor activity. Thus, in the alert rhesus monkey, darkness neurons may be more related to fixation or saccades than to luminance. Consistent with this possibility, darkness neurons were encountered caudal to the PON in a region that could correspond to the fixation region of the anterior superior colliculus (Munoz and Wurtz 1993).

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