

Single-unit responses to whole-field visual stimulation in the pretectum of *Rana pipiens*

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Abstract

Visually responsive single units were recorded from the pretectal region that includes the large-celled nucleus lentiformis mesencephali (nLM) in the leopard frog, *Rana pipiens*. During monocular stimulation of the contralateral eye, 60 single units responding to movement of a large-field, random-dot pattern were quantitatively analyzed using horizontal and vertical directions at each of four pattern velocities (0.4–40°/s). All units were spontaneously active, motion sensitive, and the majority showed 'on'-'off' responses. Several different response profiles were observed, including velocity-sensitive units with peak response at 10°/s, most of which showed directional selectivity, and speed-sensitive units that showed increasing spike frequencies as pattern velocity increased, but little or no directional selectivity. About one-third of all units analyzed were direction-selective, and 55% of those responded optimally to the temporal-to-nasal (T-N) direction of motion. T-N units were recorded primarily from an area that lies dorsolaterally between nLM and the optic tectum, in the 'peri-nLM' region. The pronounced monocular optokinetic nystagmus (OKN) response asymmetry that occurs in anurans appears to be reflected in the response profiles of the T-N direction selective units.

Keywords: Pretectum; Optokinetic nystagmus; Amphibian visual system; Nucleus lentiformis mesencephali; Direction-selective neurons; Motion-sensitive neurons

Optokinetic nystagmus (OKN) is a reflexive, repetitive response of the head and eyes which serves to stabilize the retinal image during large-field movements of the visual field. The pretectal nucleus lentiformis mesencephali (nLM) of non-mammals and its mammalian homolog, the nucleus of optic tract (NOT), are essential for the mediation of horizontal optokinetic nystagmus (hOKN) [3,6,7,11,12,22–24,29,31]. Both non-mammalian vertebrates and mammals with laterally placed eyes show an asymmetric hOKN response during monocular stimulation such that the temporo-nasal (T-N) direction is more effective in eliciting hOKN than the naso-temporal (N-T) direction [4,5,10,18,32]. Mammals with frontally placed eyes (cats, monkeys and humans) also show an asymmetric hOKN during the early postnatal period.

Both direction- and velocity-sensitive neurons have

been recorded in the neural circuitry that mediates OKN. In mammals, neurophysiological studies have shown that the NOT contains directionally selective neurons that are sensitive to large-field stimulation and respond best to T-N pattern motion during monocular stimulation [7,8,19,20]. In pigeons, Winterson and Brauth [35] described nLM units that responded over a large velocity range (0.2–80°/s). Units were subdivided into two groups: units with low preferred velocities (1–3.3°/s) and units with high preferred velocities (>10°/s). With regard to the two major subdivisions of nLM, nLM pars magnocellularis, (LMmc) and nLM pars parvocellularis, (LMpc), units in LMmc responded best at low velocities (2–4°/s) to T-N motion; whereas units in the LMpc responded best to higher stimulus velocities (>10°/s).

In frogs, Katte and Hoffmann [21] recorded direction-specific visual neurons in the pretectal region of *Rana esculenta*, and observed both horizontal and vertical response profiles. Fite et al. [13] recorded visually respon-

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sive neurons from nLM of *Rana pipiens* that were motion sensitive, with response profiles that ranged from directional and sharply tuned, to asymmetric and more broadly tuned. No particular response bias for horizontal or T-N motion was observed in nLM. Subsequently, using the 2-deoxyglucose (2-DG) method, Fite et al. [14] demonstrated that a large portion of the pretectal region that includes and surrounds nLM showed enhanced uptake of 2-DG during either T-N or N-T monocular stimulation. Thus, no directional asymmetry in 2-DG uptake was observed in the pretectum. However, the accessory optic nucleus of the basal optic root (nBOR), which has a major efferent projection to nLM [12], showed substantial 2-DG uptake only for the N-T direction of stimulation.

In order to further clarify the response properties and location of pretectal neurons responding during whole-field motion, visually responsive single units were recorded from nLM and the surrounding pretectal region. Responses to four directions of pattern motion, at velocities from 0.4–40°/s, were analyzed during stimulation of the contralateral eye using objective criteria as described previously [13].

Adult *Rana pipiens* were anesthetized by immersion in tricaine methyl sulfonate solution (1:500 concentration). The dorsal surface of the brain was surgically exposed and the dura mater carefully removed. The frog was immobilized with succinylcholine (0.05 mg/g body weight), and anesthesia was maintained throughout the entire recording session by periodic rinses of the body surface with tricaine solution. The pretectal region was localized using stereotaxic coordinates obtained from an existing series of coronal brain sections containing horseradish-peroxidase labeled retinal axons and terminals. Glass micropipettes (impedance 0.5–5.0 M Ω at 1000 Hz) were filled with 2.5 M NaCl and 50 mM CoCl₂ solution [34].

During the recording session, the frog was placed in a stereotaxic apparatus attached to a movable platform that could be rotated to align the receptive field of each single unit with the center of a tangent, rear-projection screen (Polacoat vinyl, 1000 × 1000 cm) located 25 cm from the frog's corneal surface. The stimulus was presented using a film-strip projector modified to drive a continuous loop of 35 mm film using a variable speed, reversible motor. The projector was optically equipped with a Dove prism that could be rotated to produce either horizontal or vertical directions of pattern motion [13].

Four directions of pattern motion were presented to each unit in the order: nasal-to-temporal (N-T); temporal-to-nasal (T-N); ventral-to-dorsal (V-D); dorsal-to-ventral (D-V), at each of four pattern velocities (0.4°, 2°, 10°, and 40°/s). To enable comparisons of baseline activity with visual responses to stimulus pattern motion, several 10 s intervals of spontaneous activity were recorded for each unit in the presence of the illuminated, blank screen. An equivalent amount of baseline activity was also recorded from each unit to the stationary, random-dot pat-

tern. Successive presentations of the moving stimulus pattern were separated by one-minute interstimulus intervals. Response activity was conventionally preamplified, displayed on a dual-beam storage oscilloscope and simultaneously recorded on a four-channel tape recorder (TASCAM 22-4) for off-line analysis.

Single-unit recordings were obtained during monocular stimulation of the contralateral eye; the ipsilateral eye was covered with an opaque occluder. At the conclusion of the recording session, a positive current was used to eject a small quantity of Co²⁺ from the electrode tip in order to localize the recording site in approximately one-quarter of the units analyzed [26]. The brain was removed from the skull and immersed in a saline solution containing ammonium sulfide (10%) for 30 min to develop the black, cobalt-sulfide precipitate marking the recording site. Following paraffin embedding, 10 μ m-thick coronal, serial sections were cut and saved from caudal-thalamic to mid-mesencephalic levels and were counterstained with cresyl violet (Fig. 1).

Action potentials were analyzed off-line using a window-discriminator module interfaced with an analog-to-digital signal converter that presented digitized spikes to an IBM-compatible computer for quantitative analysis. For each unit, the number of spikes occurring in each 10 s baseline condition was counted and stored. A response to pattern motion was defined as an increase in activity level that exceeded the baseline rate by at least 50%. Spike-frequency histograms were generated using software developed previously [13].

Responses were recorded and analyzed from a total of 60 single units. Twenty-seven units were recorded from nLM; a second group of 24 units were recorded from the region immediately dorso-lateral to nLM, designated as the peri-nLM region. Nine units were recorded from the pretectal gray (Fig. 1). All units were spontaneously active (2–12 impulses/s), and most responded to 'on'-'off' changes in ambient illumination. All units responded over the full range of stimulus velocities tested (0.4° to 40°/s). In 19 of 24 units recorded from the peri-nLM

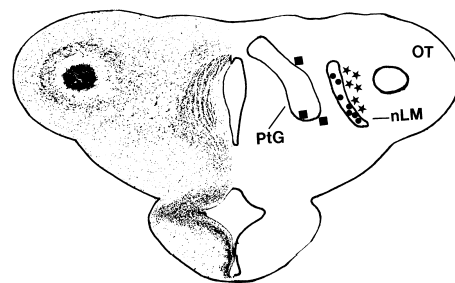


Fig. 1. Left, Coronal section through the anterior mesencephalon and pretectal region stained with cresyl violet. Right, Location of single-unit recording sites in the pretectal nucleus lentiformis mesencephali (nLM) (dots), peri-nLM region (stars), and pretectal gray (PtG) (squares), respectively. (OT, optic tectum).

region, spike frequencies increased as velocity increased from 0.4° to 10°/s, with a maximum response at 10°/s. A second type of speed-sensitive unit was recorded primarily from nLM. In 23 units, spike frequencies increased as pattern velocity was increased from 0.4° to 40°/s, regardless of the direction of pattern motion (Fig. 2). None of these units showed strong directional preferences.

Unit responses were classified as direction-selective (DS) if the response to one stimulus direction was at least 50% greater than to the other three stimulus directions for any given pattern velocity. Twenty units recorded (33% of total) met this criterion and most of these showed their strongest directional preference at 10°/s (Figs 3 and 4). Twelve units responded best to horizontal motion, while eight responded best to vertical motion. Eleven units (55% of DS units) responded optimally to T-N pattern motion, and nine of these units were least responsive to N-T pattern motion. Only one unit responded best to N-T stimulation. With vertical motion, five units responded best to V-D and three units to D-V stimulation. DS units were recorded primarily from the peri-nLM region that lies dorsolaterally, between nLM and the optic tectum. An additional nine units were recorded more medially, in or near the pretectal gray. Three of these units responded optimally to a stimulus velocity of 4°/s, but showed no clear directional selectivity.

In general, the results of the present study are consistent with a previous 2-deoxyglucose study indicating that a large region of the pretectum is strongly activated during hOKN [14]. Fite et al. [13] found that units recorded from nLM showed no bias for either horizontal or T-N pattern motion, and postulated that T-N neurons might occur in other regions of the pretectum. In the present study, T-N direction-selective responses were recorded from an area that lies dorsolaterally between nLM and the optic tectum, termed the ‘peri-nLM’ area. Units optimally responsive to T-N motion also have been recorded from the dorsal term-

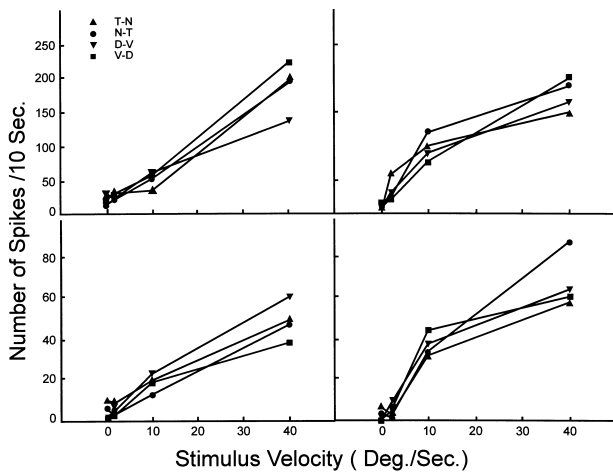


Fig. 2. Single-unit response profiles recorded from four representative, speed-sensitive units for each of four directions of stimulus motion and four stimulus velocities recorded from nLM.

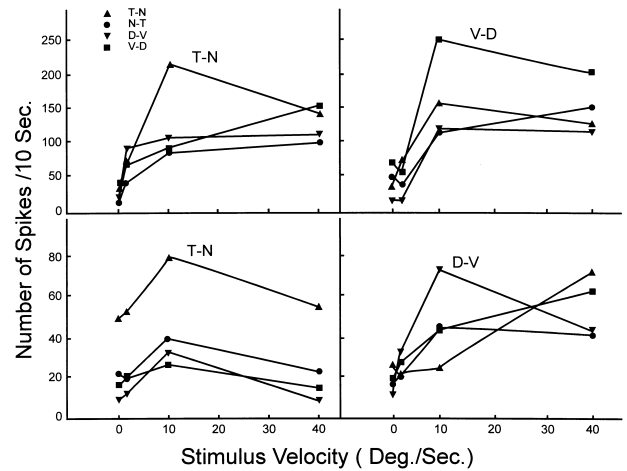


Fig. 3. Responses recorded from four representative, direction-selective, peri-nLM units for each of four directions of stimulus motion and four stimulus velocities.

inal nucleus (DTN) in the anterior mesencephalon of rabbits [30]; however, a homologous nucleus has not been identified in anurans.

A number of studies in both mammalian and non-mammalian species have suggested that some directionally selective units respond best to slower pattern velocities (so-called ‘retinal slip’ neurons) [14,20,24,27]. The results of the present study are consistent with an earlier study in frog [13], showing that 80% of directional units recorded from the pretectum are speed sensitive. Velocity- or speed-sensitive pretectal units also have been reported in a wide range of species, including the pigeon nLM (80% of total) [35], turtle nLM and nBOR [28], in the NOT of cats [19,20] as well as awake, behaving primates (90% of

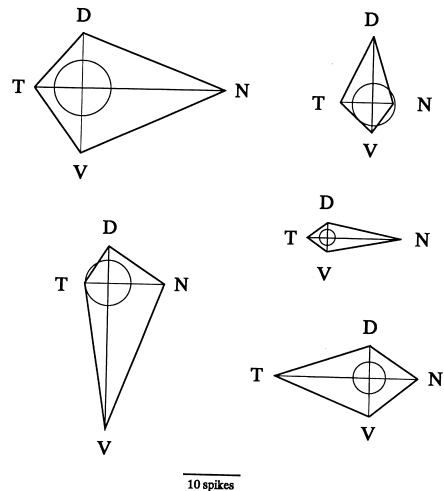


Fig. 4. Response profiles for five direction-selective units at a stimulus velocity of 10°/s. The inner circle shows the spontaneous activity level for each unit; line length represents response magnitude for each direction of pattern motion (calibration bar, 10 spikes). Temporal (T), nasal (N), ventral (V) and dorsal (D) directions of motion (i.e. temporal-to-nasal motion is from left to right).

total) [25]. In the frog retina, Class 2 and Class 3 ganglion cells retina show linearly increasing spike frequencies to movement of individual small contours [15]; however, these retinal afferents show rapid habituation to repeated or sustained stimulation such as whole-field motion that elicits OKN.

The origin of directionally selective neuronal responses in the frog pretectum remains unclear. Afferents both from the retina and from nBOR may contribute to DS response profiles in the frog pretectum. Backstrom et al. [2] reported that 17% of frog retinal ganglion cells show some degree of directional selectivity; however, the degree of directional tuning for each ganglion cell is quite broad, generally encompassing a sector of 180° or more. By comparison, the response profiles of DS units recorded in the frog pretectum and nBOR are more narrowly tuned [13,17]. Direction-selective neurons are considered to be essential components of the neural circuitry that controls OKN; however, considerable evidence from such diverse sensorimotor control systems as locust leg movements, mammalian eye saccades and visually guided reaching movements indicates that the directional control of movement is mediated not only by highly tuned directional neurons, but involves a larger population of neurons with diverse response profiles [1,16,33]. New techniques that permit direct recording of activity in multiple-neuron ensembles during behavioral events should allow the dynamic properties of a neuronal ensemble code to be more fully understood than is possible with sequential analysis of changes in the response rate of single neurons [9].

In conclusion, the sensory control of hOKN in frogs appears to utilize both speed- and direction-selective neurons within a complex circuit that involves the retina, nLM, the peri-nLM region, as well as the accessory optic nucleus, nBOR. The T-N, directionally selective units recorded from the peri-nLM region in the present study reflect the strong, behavioral asymmetry that occurs during monocular, hOKN stimulation in unanesthetized animals. The synaptic organization that underlies the functional organization of the hOKN neural circuitry and the T-N response asymmetry, in particular, remains to be elucidated.

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- [1] Altman, J. and Kien, J., Many neurons make light work?, *New Sci.*, 139 (1993) 34–38.
- [2] Backstrom, A.C., Hemila, S. and Reuter, T., Directional selectivity and colour coding in the frog retina, *Med. Biol.*, 56 (1978) 72–83.
- [3] Ballas, I. and Hoffmann, K.-P., A correlation between receptive field properties and morphological structures in the pretectum of the cat, *J. Comp. Neurol.*, 238 (1985) 417–428.
- [4] Birukow, G., Untersuchungen über den optischen Drehnystagmus und über die Sehschärfe des Grasfrosches (*Rana temporaria*), *Z. Vergl. Physiol.*, 25 (1937) 92–142.
- [5] Collewijn, H., Optokinetic eye movements in the rabbit: input-output relations, *Vis. Res.*, 9 (1969) 117–132.
- [6] Collewijn, H., Oculomotor areas in the rabbit's midbrain and pretectum, *J. Neurobiol.*, 6 (1975) 3–22.
- [7] Collewijn, H., Direction-selective units in the rabbit's nucleus of the optic tract, *Brain Res.*, 100 (1975) 489–508.
- [8] Collewijn, H., Sensory control of optokinetic nystagmus in the rabbit, *Trends Neurosci.*, 11 (1980) 277–280.
- [9] Deadwyler, S.A. and Hampson, R.E., Ensemble activity and behavior: what's the code?, *Science*, 270 (1995) 1316–1318.
- [10] Dieringer, N. and Precht, W., Compensatory head and eye movements in the frog and their contribution to stabilization of gaze, *Exp. Brain Res.*, 47 (1982) 394–406.
- [11] Fite, K.V. and Montgomery, N.M., Neuronal correlates of optokinetic nystagmus (OKN) in the amphibian mesencephalon: a functional analysis, *Neuroscience*, 7 (1982) 69.
- [12] Fite, K.V., Pretectal and accessory-optic visual nuclei of fish, amphibia and reptiles: theme and variations, *Brain Behav. Evol.*, 26 (1985) 71–90.
- [13] Fite, K.V., Kwei-Levy, C. and Bengston, L.C., Neurophysiological investigation of the pretectal nucleus lentiformis mesencephali in *Rana pipiens*, *Brain Behav. Evol.*, 34 (1989) 164–170.
- [14] Fite, K.V., Bengston, L.C., Taggart, G., Montgomery, N. and Tyler, C., Metabolic correlation of optokinetic stimulation in the central visual system of the frog *Rana pipiens*, *J. Comp. Neurol.*, 316 (1992) 459–466.
- [15] Gaillard, F. and Garcia, R., The velocity function of ipsilateral visual units in the frog optic tectum: comparison with retinal ganglion cells, *Neurosci. Lett.*, 65 (1986) 99–103.
- [16] Georgopoulos, A.P., Schwartz, A.B. and Kettner, R.E., Neuronal population coding of movement direction, *Science*, 233 (1986) 1416–1419.
- [17] Gruberg, E.R. and Grasse, K.L., Basal optic complex in the frog (*Rana pipiens*): a physiological and HRP study, *J. Neurophysiol.*, 51 (1984) 998–1010.
- [18] Hess, B.J.M. and Precht, W., Reber, A. and Cazin, L., Horizontal optokinetic ocular nystagmus in the pigmented rat, *Neuroscience*, 15 (1985) 97–107.
- [19] Hoffmann, K.-P. and Schoppmann, A., A quantitative analysis of direction-specific responses in the cat's nucleus of the optic tract, *Exp. Brain Res.*, 51 (1981) 236–246.
- [20] Hoffmann, K.-P. and Distler, C., Quantitative analysis of visual receptive fields of neurons in nucleus of the optic tract and dorsal terminal nucleus of accessory optic tract in Macaque monkeys, *J. Neurophysiol.*, 62 (1989) 416–428.
- [21] Katte, O. and Hoffmann, K.-P., Direction specific neurons in the pretectum of the frog (*Rana esculenta*), *J. Comp. Physiol.*, 140 (1980) 53–57.
- [22] Lazar, G., Alkonyi, B. and Toth, P., Re-investigation of the role of the accessory optic system and pretectum in the horizontal optokinetic nystagmus of the frog. Lesion experiments, *Acta. Biol. Hungarica*, 34 (1983) 385–393.
- [23] McKenna, O.C. and Wallman, J., Accessory optic system and pretectum of birds: comparisons with those of other vertebrates, *Brain Behav. Evol.*, 26 (1985) 91–116.
- [24] Montgomery, N.M., Fite, K.V., Taylor, M. and Bengston, L.C., Neural correlates of optokinetic nystagmus in the mesencephalon of *Rana pipiens*: a functional analysis, *Brain Behav. Evol.*, 21 (1982) 137–150.
- [25] Mustari, M.J. and Fuchs, A.F., Discharge patterns of neurons in the pretectal nucleus of the optic tract (NOT) in the behaving primate, *J. Neurophysiol.*, 64 (1990) 77–90.
- [26] Pitman, R.M., Tweedle, C.D. and Cohen, M.J., Branching of central neurons: intracellular cobalt injection for light and electron microscopy, *Science*, 172 (1972) 412–414.
- [27] Precht, W. and Strata, P., Pathways mediating optokinetic responses of cat's vesicular neurons, *Neuroscience*, 3 (1979) S351.

- [28] Rosenberg, A.F. and Ariel, M., A model for optokinetic eye movements in turtles that incorporates properties of retinal-slip neurons, *Vis. Neurosci.*, 12 (1996) 375–383.
- [29] Simpson, J.I., Giolli, R.A. and Blanks, R.H.J., The pretectal nuclear complex and the accessory optic system. In J. Buttner-Ennever (Ed.), *Neuroanatomy of the Oculomotor System*, Elsevier, Amsterdam, 1988, pp. 335–364.
- [30] Simpson, J.I., Soodak, R.E. and Hess, R., The accessory optic system and its relation to the vestibulocerebellum, *Prog. Brain Res.*, 50 (1979) 715–724.
- [31] Simpson, J.I., The accessory optic system, *Annu. Rev. Neurosci.*, 7 (1984) 13–41.
- [32] Sontheimer, H. and Hoffmann, K.-P., Horizontal optokinetic reflex in light and dark-reared Israeli gerbils (*Meriones tristrami*), *Exp. Brain Res.*, 66 (1987) 440–444.
- [33] Sparks, D.L., Neuronal cartography: sensory and motor maps in the superior colliculus, *Brain Behav. Evol.*, 31 (1988) 49–56.
- [34] Wang, S.R., Yan, K. and Wang, Y.T., Visual field topography response in the frog's nucleus isthmi, *Neurosci. Lett.*, 23 (1981) 37–41.
- [35] Winterson, B.J. and Brauth, S.E., Direction-selective single units in the nucleus lentiformis mesencephali of pigeon (*Columba livia*), *Exp. Brain Res.*, 60 (1985) 215–226.