Visual Neuroscience (2006), **23**, 209–219. Printed in the USA.
Copyright © 2006 Cambridge University Press 0952-5238/06 $16.00
DOI: 10.1017/S0952523806232061

Responses of the teleostean nucleus isthmi to looming objects and other moving stimuli

SHAWN P. GALLAGHER[1] AND DAVID P.M. NORTHMORE[2]

[1]Department of Psychology, Millersville University, Milersville, Pennsylvania
[2]Department of Psychology, University of Delaware, Newark, Delaware

(RECEIVED November 30, 2004; ACCEPTED September 29, 2005)

Abstract

Visually evoked extracellular neural activity was recorded from the nucleus isthmi (NI) of goldfish and bluegill sunfish. When moving anywhere within the right eye's visual field, three-dimensional checkered balls or patterns on a computer screen evoked bursts of spikes in the left NI. Object motion parallel to the longitudinal body axis gave responses that habituated markedly upon repetition, but movement into recently unstimulated regions of the visual field gave vigorous responses. Thus, while NI's response is not visuotopic, its habituation is. An object approaching the animal's body generated a rising spike density, whereas object recession generated only a transient burst. During the approach of a checkered stimulus ball, average NI spike density rose linearly as the ball-to-eye distance decreased and at a rate proportional to the ball's speed (2.5–30 cm/s). Increasing ball size (2.2–9.2 cm) did not affect the rate of activity rise at a given speed, but did increase overall activity levels. NI also responded reliably to expanding textures of fixed overall size. The results suggest that NI signals changes in motion of objects relative to the fish, and estimates the proximity of approaching objects.

Keywords: Nucleus isthmi, Optic tectum, Teleost, Looming, Motion

Introduction

In nonmammalian vertebrates, the midbrain tectum is reciprocally connected to the paired nuclei isthmi (NI) in the dorsolateral tegmentum (for reviews of NI anatomy and physiology, see Vanegas & Ito, 1983; Gruberg, 1983; Wang, 2003); in mammals the homologous structures are the parabigeminal nuclei (Graybiel, 1978; Cui & Malpeli, 2003). In birds and reptiles, each NI is a complex of several subnuclei, but in fishes and amphibians it appears to be a unitary structure. In general, NI's interconnections with tectum are topographically organized, deriving a major input from the ipsilateral tectal lobe, and returning a projection to it. In most species studied, each NI also projects to the contralateral tectal lobe. Electrophysiological recordings from NI in various species have revealed visually responsive neurons as might be expected from the substantial input it receives from tectum, but its role in vision is still obscure.

The contralateral isthmo-tectal projection allows the combination of visual information from the two eyes within tectum. This pathway has been much studied in amphibians because of its potential role in the formation of binocular units in tectum that could be used for judging depth during prey capture (Glasser & Ingle, 1978; Grobstein et al., 1978; Grobstein & Cromer, 1983; Gruberg & Udin, 1978; Wiggers & Roth, 1991). While the function of NI as an intertectal relay for binocularity has been challenged on several grounds, among them the existence of a faster, ipsilateral retinal input to tectum (Wang, 2003), the NI relay, because of its considerable time delay, might nevertheless play a part in determining the three-dimensional trajectories of fast moving objects (Wiggers & Roth, 1991). Others suggest NI mediates inhibitory interactions between the tectal lobes (Wang, 2003), or that it acts as a winner-take-all mechanism enabling an animal to attend selectively to events or objects (Gruberg et al., 1991; Wang & Frost, 1991; Sereno & Ulinski, 1987). Consistent with these ideas are findings that ablation of NI in anurans impairs behavioral responses to threat and prey objects, and interferes with stimulus selection (Gruberg et al. 1991; Collett et al., 1987). Collectively, the amphibian studies implicate NI as a modulator of important spatial processing for visuomotor behaviors by the tectum.

In teleost fishes, most of the anatomical studies of NI have examined cyprinid and perciform species and revealed topographically arranged ipsilateral connections between NI and tectum (Grover & Sharma, 1981; Sakamoto et al., 1981; Dunn-Meynell & Sharma, 1984; Xue et al., 2001). Although some studies (Dunn-Meynell & Sharma, 1984; Brandis & Saidel, 2001; Pérez-Pérez et al., 2003) have detected a minor projection of NI to the contralateral tectal lobe, electrophysiological recordings in fish generally show that each tectal lobe, and its ipsilateral NI, are mainly concerned with processing visual information from space on the contralateral side of the animal (Northmore, 1991). Study-

Address correspondence and reprint requests to: David P. M. Northmore, Department of Psychology, University of Delaware, Newark, DE 19716-2577, USA. E-mail: northmor@udel.edu

ing both goldfish and sunfish, we found that an extracellular electrode, wherever inserted into NI, records spiking activity that is triggered by changing visual stimulation throughout the visual field of the contralateral eye (Northmore & Gallagher, 2003). This activity is unusual in that the spikes, although high in amplitude, cannot be resolved into conventional single units, and that virtually identical patterns of firing are recorded simultaneously at well-separated sites within the nucleus. Thus, it appears that NI, or a large fraction of its neurons, discharges as a unit, perhaps mediated by electrical coupling between its cells (Ito et al., 1982; Williams et al., 1983). As a result, the topography provided by the tectal input is not apparent in the NI signal, which is broadcast over the ipsilateral tectal lobe and mirrored by multiunit spiking activity in the deep layers of tectum (Northmore & Gallagher, 2003).

The visual response properties of teleost NI, in particular its whole-field responsiveness to novelty and to stimulus motion without directional selectivity, call to mind the similar properties of two identified neurons of locusts, the lobula giant movement detector (LGMD), and the cell to which it connects, the descending contralateral movement detector (DCMD) (O'Shea & Williams, 1974). These cells fire most strongly to objects that approach the eye on a collision course and probably initiate the locust's escape response (Schlotterer, 1977; Judge & Rind, 1997). We therefore wondered whether NI might show a similar preference for looming objects. The results we report do show such a preference and suggest that NI together with tectum is capable of extracting information about depth using monocular motion information. Despite the strangeness of teleost NI response properties, it may yet have some commonality of function with NI in amphibians.

Materials and methods

Preparations and neural recording

Bluegill sunfish (*Lepomis macrochirus*, 8–15 cm standard length) and goldfish (*Carassius auratus*, 8–13 cm standard length) were obtained from a local hatchery (Hiram Peoples Hatchery, New Providence, PA), maintained at 20–24°C on a 14-h light/10-h dark cycle, and fed dried goldfish pellets. All procedures were identical for both species unless otherwise indicated and were approved by the University of Delaware Institutional Animal Care and Use Committee.

Before electrophysiological recording, fish were anesthetized by immersion in a solution of MS222 (Sigma Chemical, St. Louis, MO) buffered to neutral pH. In some experiments, fish were paralyzed with an injection of 0.2 mg Flaxedil (Davis & Geck, Pearl River, NY) into the dorsal musculature. In other experiments, fish were immobilized by cutting the spinal cord. We saw no obvious differences between the two methods of immobilization on the results. Fish were then wrapped in wet paper towels and placed upright in a V-block holder. A stainless-steel mouth tube supplied a continuous stream of aerated water during recordings and served as a connection to electrical ground. The midbrain was exposed by opening the cranium and aspirating the overlying perimeningeal tissue. The wound margins were treated with a local anesthetic (Lidocaine gel, 2%). The myopia of the eye in air was corrected with hemispherical contact lenses made of acrylic plastic in two sizes, one designed for goldfish, the other for sunfish (Northmore, 1989, 1991). The inner concavity of the lens was gently applied to the cornea and any space between the cornea and lens was filled by a water stream continuously fed by a small tube leading from a reservoir.

Neural activity was recorded extracellularly from the brain using stainless-steel microelectrodes (FHC, Bowdoinham, ME) with tip resistances of 0.2–1 MΩ. Activity was amplified with a bandpass of 300 Hz–3 kHz and fed to a leaky integrator that performed a full-wave rectification and integration with a time constant of 15 ms; the resulting signal, which represented a running average of total spiking activity, was digitized at 200 Hz and stored for offline analysis.

To record from NI, a microelectrode was positioned above the surface of the left optic tectum 1.0–1.3 mm lateral to the midline at the rostrocaudal level of the anterior margin of the cerebellar corpus for *Lepomis*, and slightly caudal to the anterior margin of the cerebellar corpus for *Carassius*. Visually evoked spiking activity characteristic of NI (Northmore, 1991) was obtained at a depth of approximately 1500–1800 μm in both species.

Electrode tip positions were marked by electrolytic lesions made with 5-μA positive current applied for 10 s. At the conclusion of experiments, the gills of the fish were perfused with a 1:5000 solution of MS222 and the brains were removed, immediately frozen in isopentane at −30°C, and stored at −20°C for histology.

Visual stimulation

Moving visual stimuli were presented to the right eye using a variety of methods. In some experiments, real objects were moved in three-dimensional (3-D) space; in others, moving objects were simulated by two-dimensional (2-D) patterns generated on a computer monitor.

In experiments where real objects were moved manually, the fish's eye was centered in a hemicylinder (axis vertical, 30-cm diameter, 40-cm height) covered on the interior with a black-and-white checkerboard pattern of squares subtending 4 deg of visual angle, and illuminated from above by a tungsten lamp providing an average background radiance of 30 μW/cm^2/sr. Responses were evoked by a ball (1.5- or 3-cm diameter) painted black and white in a checkered pattern that was moved in the horizontal plane through the right eye using rods that ran through holes in the checkered cylinder. The ball typically traveled 10 cm in each advance or retreat, its position being transduced by an attached search coil moving in an alternating magnetic field. The position signal was sampled simultaneously with neural activity.

Greater control over position and speed was achieved by using an analog *X*–*Y* plotter (Coulter Electronics, Hialeah, FL) controlled by a computer to move stimulus balls (2.2–9.2 cm diameter), typically checkered in black and white, in the horizontal plane through the center of the right eye. A baffle below the eye ensured that the plotter's moving parts were not seen. The apparatus and fish were enclosed by walls of light gray cardboard with an average background radiance of about 30 μW/cm^2/sr.

Simulated 3-D motion was generated by images presented on a VGA or LCD monitor placed 10–20 cm from the fish's right eye. In some experiments, expanding and contracting circles and lines one pixel wide on a gray background were used to mimic the edges of a looming stimulus; in others, expanding and contracting checkerboards were programmed.

Results

Looming versus rostro-caudal movement

Fig. 1 shows recordings made from the left NI of two *Lepomis*. In both experiments, a checkered stimulus ball (3.7 cm diameter,

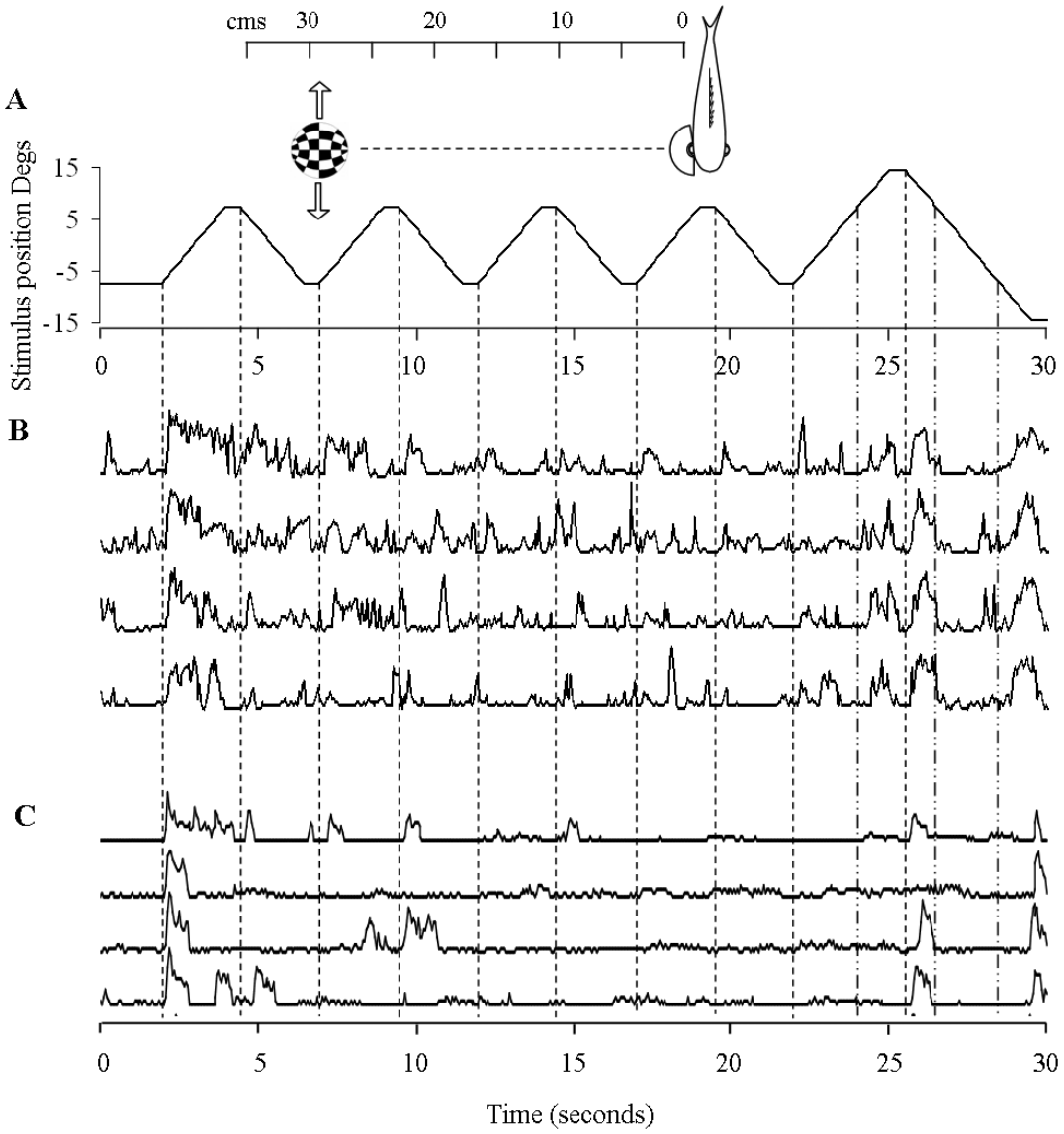

Fig. 1. NI responses of *Lepomis* to repetitive movements parallel to the body axis. A: Fish with a contact lens on the right eye. The 3.7-cm checkered ball was moved parallel to the body axis at a distance of 39 cm from the eye. The graph shows the ball's position over the 30 s of each recording. The ball started at a rostral position (−7.3 deg) and moved at 5 cm/s to a caudal position (+7.3 deg), paused for 0.5 s, returned to its starting position, and paused for another 0.5 s. This was repeated four times, followed by a final excursion to ± 14.4 deg. B: Shown from top to bottom, are four successive records of integrated activity from the left NI. Recordings were initiated at 30-s intervals. Dashed lines show the start of each ball movement; dot-dash lines show when the ball entered or left habituated regions of visual field. C: NI activity in another specimen of *Lepomis* that was less spontaneously active. Fig. 2 shows responses of this fish's NI to advance and retreat.

5.4-deg subtense) moved along a rostro-caudal path, parallel to the body axis, 39 cm from the eye in the horizontal plane of the right visual field at a constant speed of 5 cm/s or about 7 deg/s. Fig. 1A shows the position of the stimulus ball executing four to-and-fro movements of ± 7.3 deg with 0.5-s pauses between movements, followed by one larger to-and-fro excursion to ± 14.4 deg. Figs. 1B and 1C show integrated records of NI activity from the two fish to four successive repetitions of the movement initiated at about 30-s intervals.

During the very first movement, NI discharged continuously. Thereafter, discharges tended to occur as a transient burst at the beginning of motion, and with a probability that fell off after a few repetitions. Following the 30-s intertrial interval, the first movement of each series was the most effective. During the final long excursion, when the ball moved to new regions of the visual field, NI tended to respond again. In the records of Fig. 1A, these responses occurred as the ball moved within the new field region. In the second fish, whose NI was less active spontaneously, responding occurred after the ball had changed direction and moved in the new field region. Thus, NI responding habituated to repeated stimulation, but habituation was specific to recently stimulated regions of visual field.

Fig. 2 shows NI activity in a *Lepomis* during a series of movements of the 3.7-cm diameter checkered ball, first toward the

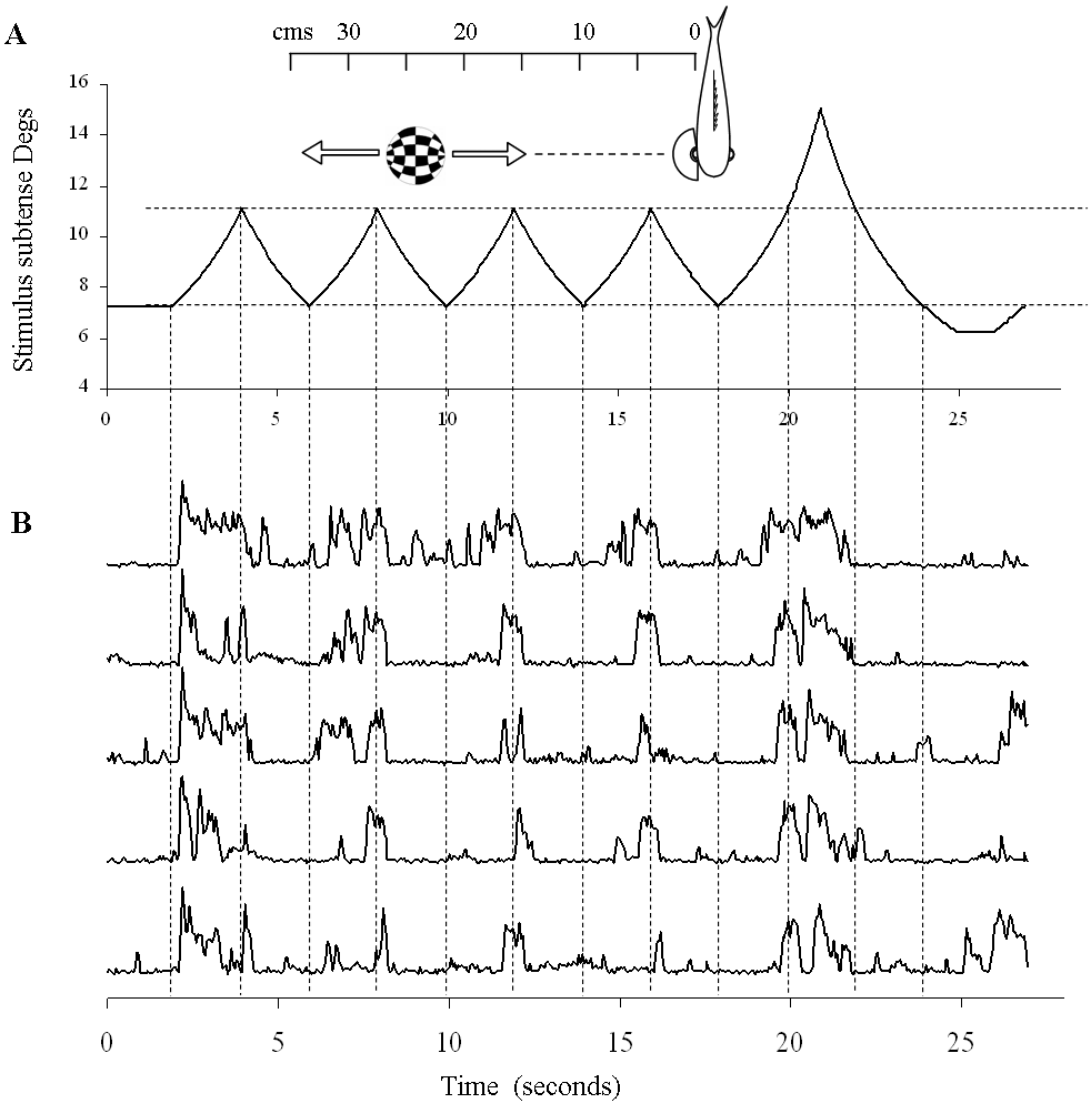

Fig. 2. NI responses of *Lepomis* to repetitive movements normal to the body axis. Data from the fish of Fig. 1C. A: The 3.7-cm diameter checkered ball oscillated between 29 and 19 cm from the right eye four times. It then moved to 14 cm followed by a move to 34 cm from the eye. Speed of ball was 5 cm/s. The graph shows the angular subtense at the eye in degrees during the movements. B: Shown from top to bottom, are five successive records of integrated activity from the left NI. Recordings were initiated at 30-s intervals.

eye and then away from the eye. At the start of the first approach of each series there was a burst of activity that tended to persist during the remainder of the movement. Subsequent approach movements gave less and more delayed activity but the nucleus nearly always responded as the ball neared the eye. Approach responses failed occasionally, usually after several repetitions. When the ball retreated from the eye, NI typically responded with a brief burst at the start of movement, and was relatively inactive during the remainder of the retreat (see also Figs. 4, 5, & 9). In the experiment of Fig. 2, after four approaches and retreats between 29 and 19 cm from the eye, the stimulus ball moved to 14 cm from the eye, followed by a retreat to 34 cm. There was a brief pause in the response when the ball reached the point of reversal on the previous four approaches, but resumed strongly while the ball was closer than the previous four closest approaches. Activity was low on the subsequent long retreat.

A separate series of experiments was done to compare NI responses in *Carassius* and *Lepomis* to parallel and approach-retreat movements using a 3-cm checkered ball moved manually at 40 cm/s (max) against the background of the checkered cylinder. In Fig. 3, and in subsequent bar-graph figures, NI response amplitudes were quantified by determining a mean baseline of integrated activity for a period before stimulation and subtracting it from the mean activity during stimulus motion. Responses are given as percentages of the largest response recorded at a given electrode site. Fig. 3 summarizes the results from three animals of each species showing that approach generated larger NI responses than did parallel movement ($P < 0.01$), and that there were no differences between rostro-caudal and caudo-rostral movement ($P > 0.05$). Neither were there significant differences between the two species ($P > 0.05$).

To control for the possibility that NI's approach and retreat responses were due to overall brightening or dimming in the visual field, we compared responses to a solid black and a solid white ball (3.8-cm diameter) moved (5 cm/s) toward and away from the eye of a *Lepomis* viewing against a gray background. The black-and-

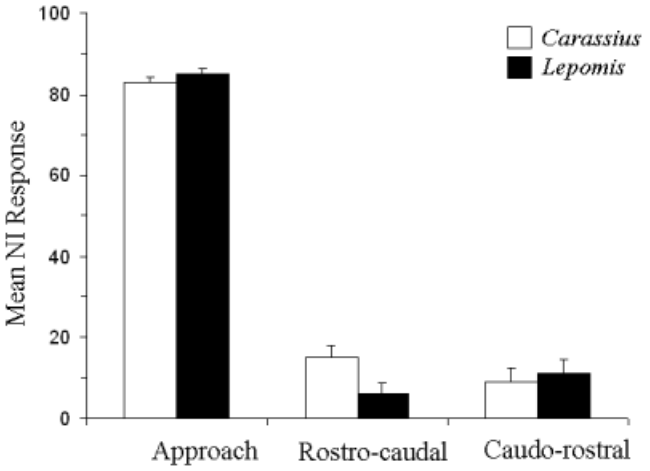

Fig. 3. NI responses in three *Carassius* and three *Lepomis* to a 3-cm checkered ball approaching the eye normal to the body axis or moving parallel to the body axis. Bars show the means (+ 1 S.E) of NI activity integrated during movement. During approaches, the ball moved between 13.5 and 3.5 cm from the eye; during parallel movements it traveled 10 cm rostro-caudally or caudo-rostrally at a distance of 10 cm from the eye. Maximum speed was 40 cm/s. Each mean was compiled from 90 responses (30 from each animal).

white balls evoked a very similar pattern of NI responding as did the same sized checkered ball (Fig. 4, 2nd record). Average amplitudes evoked by the black-and-white balls were not statistically different ($P > 0.1$).

Effect of stimulus speed

NI responds to approach over a wide range of stimulus speeds. Objects moved by hand at 100 cm/s evoked intense responses from NI when directed toward the eye, whereas the corresponding retreat evoked only a short burst of activity. To study responses to more controlled and uniform speeds of approach and retreat, checkered balls were moved on the *X–Y* plotter in the horizontal plane through the eye along paths perpendicular to the longitudinal body axis in six specimens of *Lepomis*. In a typical experiment illustrated in Fig. 4, a 3.8-cm ball advanced and retreated at different speeds between positions 39 and 9 cm from the eye. The graphs show integrated NI activity averaged over seven repetitions of the movement cycle. The start of an advance usually evoked some bursting activity from NI. At a later time during the advance, activity started to rise. After averaging several records together, this rise could be seen to form a linear ramp, reaching a maximum that occurred just after the ball stopped moving. The maximum varied little with speed of approach, and the rates of rise, shown by the straight-line regressions fitted to the data were directly proportional to the speed of approach (Fig. 4 inset graph). When the ball came to rest after an approach, the activity decayed to baseline levels, the decay occurring more slowly after high-speed approaches. The retreat phase of movement generated a peak of activity that was smaller and briefer than the activity generated on approach, and with a latency (200–500 ms) that decreased with speed. Analysis of data from three other *Lepomis* confirmed that NI linearly ramps up its activity as a stimulus ball approaches.

Effect of stimulus size

To study the effect of stimulus size, the responses of NI in *Lepomis* were recorded to checkered balls of different diameters (2.2–9.2 cm) moving at constant speed. The stimulus balls approached and retreated along a horizontal line through the right eye perpendicular to the body axis. Fig. 5 shows the activity of NI to balls moving at 5 cm/s averaged over 4–8 trials at each ball size, using a different specimen of *Lepomis* than the one used for Fig. 4. To allow habituation to dissipate, 30–45 s were allowed to elapse between each movement. The start of approach evoked bursts of activity, followed by a linear ramp-up in activity; cessation of movement saw a decay of activity to baseline; and retreats evoked reliable bursts only at the start of movement. These results resemble those shown in Fig. 4, except that the slope of the activity ramp-up appeared to be roughly constant across all sizes of stimulus ball. The ramp-up started later for smaller balls and achieved generally lower levels of activity compared to larger balls. Results similar to those in Fig. 5 were obtained in three other specimens of *Lepomis*.

Effect of stimulus path

A variety of straight-line paths of constant speed were programmed on the *X–Y* plotter. The effect of moving a 3.8-cm checkered ball at 5 cm/s in the right visual field of a *Lepomis* is shown qualitatively in Fig. 6. The ball moved 23 cm on each path to a point 8 cm from the eye. The left half of the figure shows activity during the approach phase for paths that (1) lay on or close to a line through the eye, (2) ran parallel to a line through the eye, and (3) crossed a line through the eye. The start of movement evoked some activity from NI but became more intense as the ball neared the eye. Fig. 6 (top) shows a field of high activity within a radius of about 15 cm of the eye; the magnitude of activity was not obviously affected by the path taken. Retreats along the same set of paths (Fig. 6, bottom) generated activity only at the start of movement.

Quantitative assessments of NI responding to hand-held stimuli at higher speeds (40 cm/s) were also done to compare *Carassius* and *Lepomis*, using three specimens of each species. A checkered ball advanced and retreated 10 cm on a line through the eye at azimuths of 45 deg, 90 deg, and 135 deg and came to rest 3.5 cm from the eye. Recordings of integrated NI activity were averaged from ten trials with each fish. The resulting mean response amplitudes are shown as bar graphs in Figs. 7B–7D. Responses to advances were always greater than responses to retreats for all three paths of motion in both species ($P < 0.01$). There was no significant effect of path azimuth ($P > 0.05$) or difference between species ($P > 0.05$).

Two-dimensional expanding images

Expanding and contracting stimuli were programmed on the screen of a computer monitor. Fig. 8 compares the mean integrated responses of NI in both *Carassius* and *Lepomis* to changes in the size of a circle and a pair of parallel vertical lines. The lines composing the stimuli were one pixel in width. The expanding and contracting circle, whether drawn in black or white on a gray background, evoked the same patterns of response from NI that the approaching and retreating checkered balls evoked (e.g. Fig. 4, 30 cm/s). However, when the pair of vertical lines was enlarged by the same amount as the circle, only a few bursts of extra activity

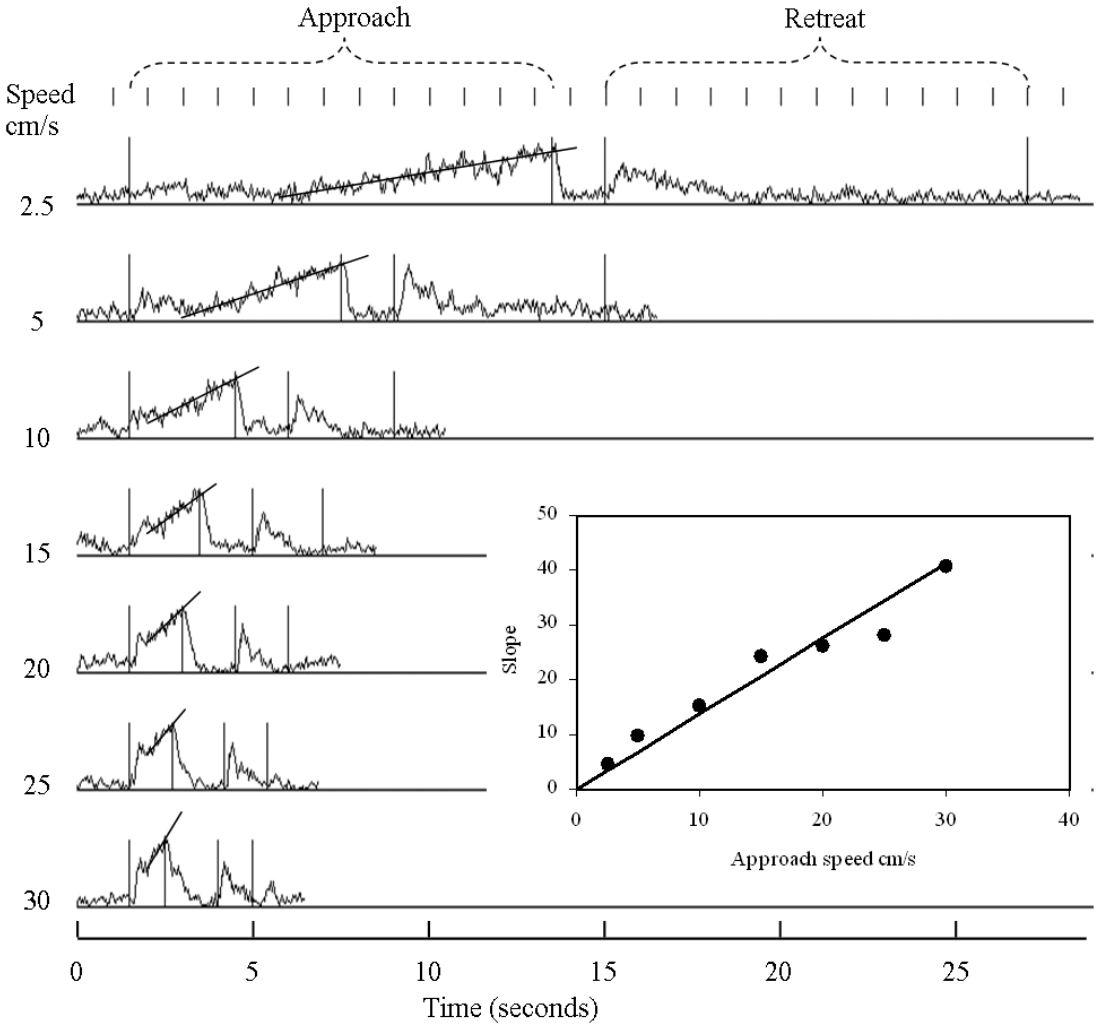

Fig. 4. Responses of NI in a *Lepomis* to different speeds of movement of a 3.8-cm checkered ball. The ball traveled at a constant rate on a horizontal path perpendicular to the body axis through the right eye, first approaching from 39 cm to 9 cm, pausing 3 s, and then retreating along the same path. The vertical lines mark the start and end of movement. Each record is an average of the integrated activity over seven trials. Linear regression lines are shown fitted to the phase of steadily increasing activity during approach. Inset graph: slope of regression lines as a function of approach speed.

were evoked compared to spontaneous. As Fig. 8 shows, the average responses to the expanding lines in both species were much less than to the expanding circle ($P < 0.01$). Again, there were no statistical differences between the responses of the two species ($P > 0.05$).

To simulate changes in distance from an extended patterned surface, a black–white checkerboard pattern was programmed to enlarge and contract on the monitor screen as though it were approaching or retreating at a constant speed. In this experiment the right eye of a *Lepomis* viewed the checkerboard through a circular window, fixing the overall size of the moving image to 20 deg of visual angle. Fig. 9 shows that an expanding pattern was effective in eliciting activity from NI, especially at the onset of motion. In this experiment, activity was sustained for most of the approach phase, but declined somewhat as the checks grew larger and fewer in numbers. Variation in the ratio of transient to sustained activity was seen in the three different specimens of *Lepomis* studied with approaching textures. Retreating textures elicited strong initial activity that declined to baseline levels before movement ceased.

Discussion

As shown previously for *Carassius* and *Lepomis* (Northmore, 1991; Northmore & Gallagher, 2003), activity recorded extracellularly from NI consisted of bursts of spiking potentials, with individual spikes varying over a range of amplitudes up to 1 mV or more. NI response was quantified, not by firing frequency because single units cannot be recorded extracellularly, but by the output of the leaky integrator giving an indication of spike density. Because NI exhibits no visuotopic organization, and because almost identical activity waveforms are recorded anywhere within the nucleus (Northmore & Gallagher, 2003), the positioning of the recording electrode within NI probably had no influence on the results.

Habituation

It was known from previous electrophysiological studies (Williams et al., 1983; Northmore, 1991; Northmore & Gallagher,

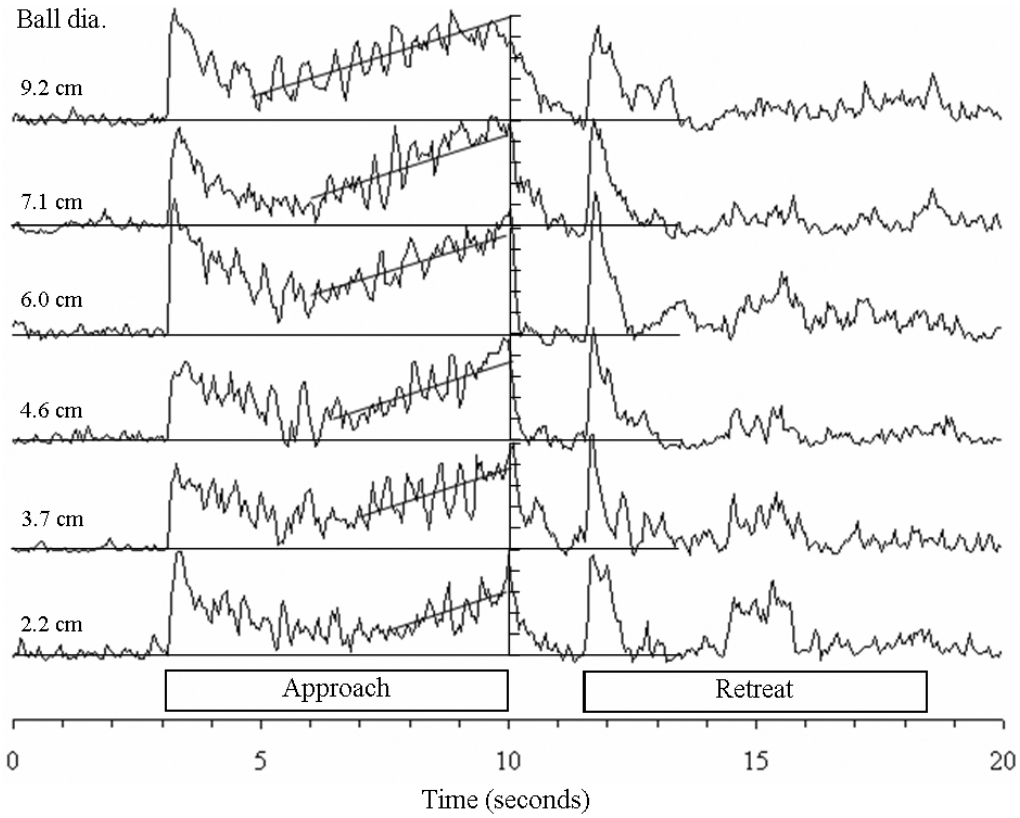

Fig. 5. Responses of NI in a *Lepomis* to checkered balls of different sizes approaching and retreating at 5 cm/s on a horizontal line through the eye at 90 deg to the body axis. Each record is an average of 4–8 repetitions. Lines of equal slope are shown fitted to the phase of steadily increasing activity during approach. Scales at the end of approach allow comparison of the final response amplitudes.

2003) that the teleost NI responds to changing visual stimulation throughout the contralateral visual field, and that moving objects are particularly effective. However, responses diminish with repetition of the stimulation. The present study, using real moving objects (Fig. 1), confirmed our previous finding with flashed LEDs (Northmore & Gallagher, 2003) that response habituation is specific to the region of visual field repetitively stimulated. One implication is that the neurons that drive NI are visuotopically arrayed and are subject to habituation. Several electrophysiological studies in the tectum of cyprinids and perciforms have found single units that habituate to repeated stimulation (O'Benar, 1976; Guthrie & Banks, 1978; Kawasaki & Aoki, 1983). The major input to NI which is excitatory and topographically arranged, comes from the ipsilateral tectal lobe, specifically the Type XIV tectal neurons (Meek, 1983) in perciforms, and both the Type XIV and Type VI neurons in cyprinids (King & Schmidt, 1993; Xue et al., 2001). Habituation may occur in these cells or the tectal circuits that involve them. Alternatively, it may be the tectoisthmic synapses that habituate. However, electrical stimulation of tectum reliably excites NI cells transynaptically (Williams et al., 1983; Northmore & Gallagher, 2003), making this latter explanation unlikely. The obverse of NI's habituation is its tendency to signal change or variation. This was seen particularly at the start of motion, or at abrupt changes in direction (Fig. 2). While habituation reduces NI responding to all kinds of stimulation, tending to overcome weak responses to nonoptimal stimuli such as light flashes (Northmore & Gallagher, 2003), responses to particularly salient events such as looming stimuli are less affected.

Proximity

Although any movement of a stimulus object may excite NI, movements approaching the fish evoked the most activity and with the greatest reliability. In an attempt to understand this response, we consider the case illustrated by Fig. 4 in which a ball of a given size approaching the eye at a range of constant speeds evoked linear ramp-ups in average spiking density as the distance between the ball and the eye diminished. The increase in NI spiking density did not resemble the increases in either of the two optical variables available to the visual system, the image size, $\theta(t)$, and its rate of expansion, $\rho(t)$, because these are both positively accelerated functions of time. The linear rise of NI activity might be explained if it were negatively related to time to collision, $\tau(t)$, which for constant approach speed is given by $\theta(t)/\rho(t)$ and declines linearly with time. Neurons in the visual system of pigeons fire when $\tau(t)$ drops to a critical value, although none responds with a linear function of this variable (Sun & Frost, 1998). However, a function such as $(k - \tau(t))$, where k is a constant, while rising linearly during approach, does not increase its slope with increasing speed. Results such as those of Fig. 4 show that (1) the slope of the activity ramp-up was proportional to the speed of a stimulus ball, and (2) the ramp-up maximum achieved at the end of the approach was approximately independent of speed. The implication of these two observations is that the instantaneous NI activity during approach estimates what we might call "proximity," a quantity which is proportional to $(k - D(t))$, where $D(t)$ is the distance of the ball to the eye.

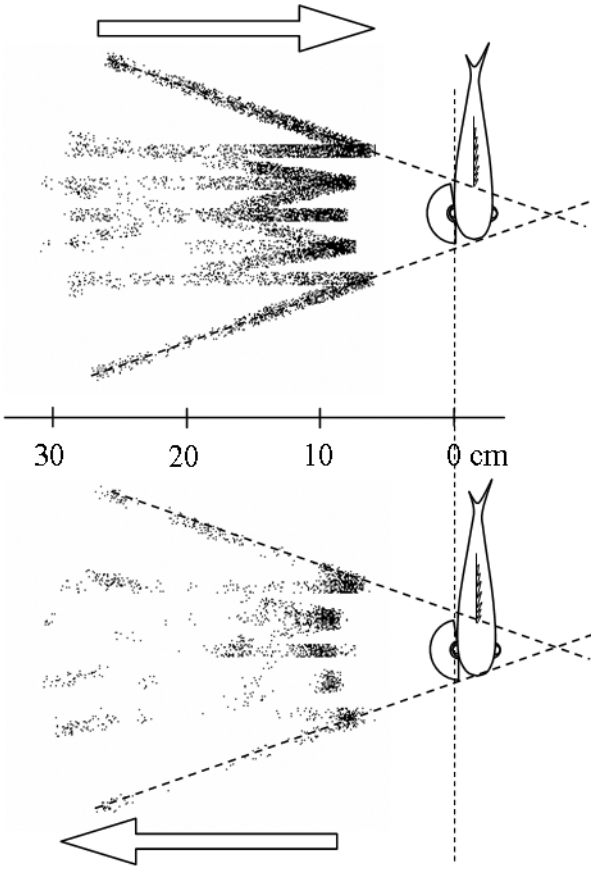

Fig. 6. Responses of NI in a *Lepomis* during various straight line paths of a 3.8-cm checkered ball approaching (left) and retreating (right) in the horizontal plane through the right eye at 5 cm/s. Responses of four repetitions are shown superimposed along each path and response magnitude is represented by dot density.

While NI could, in principle, measure the distance of an approaching ball of known size, uncertainty in ball size would throw off the measurement. Fig. 5 shows the effect of varying the sizes of approaching balls on NI response at one speed of approach. Although the ramp-ups were of similar slope, larger balls generally produced larger responses with earlier starts to the ramps—an error, if that is what it is, on the side of safety. More detailed quantitative studies are required to characterize precisely what it is that NI is responding to in an approaching stimulus.

NI was excited by objects that approached the animal along a variety of paths (Fig. 6) and not just by paths through the eye (Fig. 7). Indeed paths parallel to the body axis were also effective (Figs. 1 & 3). Expansion of just an outline or boundary of an object, such as the expanding circle (Fig. 8A) was clearly effective in eliciting typical approach/retreat responses (Fig. 8A), but outline expansion was not necessary for NI to be excited because expanding patterns of fixed overall size, in which multiple texture elements enlarged, were also very effective at eliciting the approach/retreat response (Fig. 9). Interestingly, the expanding vertical lines of Fig. 8B were ineffective, as though this pattern represented not an approaching object but a space enclosed by passing walls, as it does to our eyes. Taken together, the results suggest that NI could have a function in detecting the proximity of objects that are approaching the animal such as other fish, or the proximity of extended surfaces, such as rocks and vegetation that the animal itself approaches.

Neural mechanisms

The responses of NI to motion in *Lepomis* and *Carassius* were similar by all the measures employed in this work. We compared the two species in a previous study (Northmore & Gallagher, 2003) because, as noted above, the tectoisthmic cell types differ between cyprinids and perciforms, as do the pathways from the pretectum/thalamus to NI (King & Schmidt, 1993; Xue et al., 2001), but again, we found no significant differences between the two species.

The response properties of NI were known to resemble those of the locust LGMD in that both have a wide receptive field, both respond to motion of objects anywhere within this field, and both tend to habituate. The present results show another point of similarity in that NI responds much more strongly to approach than to retreat. There are significant differences, however. LGMD is subject to pronounced lateral inhibition that makes it respond better to small rather than large objects (Rowell et al., 1977), while NI's response tended to increase with stimulus size. LGMD is highly selective for the path of approach, responding much more strongly to objects approaching within 3 deg of a collision course with the eye (Rind & Bramwell, 1996); NI appeared to be omnidirectional for approach, responding to all paths close to the body, yet still discriminating against retreat (Fig. 7). Despite these differences, it is possible that a mechanism similar to that proposed by Rind and Bramwell (1996) to account for LGMD's preference for looming stimuli might also apply to NI's responses in teleosts. Adapting their model, we suppose that the leading edges of a moving image on the retina excite topographically mapped cells in tectum but also subject them to inhibition that is delayed with respect to the excitation. During the approach of an object, and the expansion of its retinal image, the boundary of excitation on the tectum exceeds the boundary of inhibition such that the net excitation drives NI to fire. Upon retreat of the stimulus object, the contracting boundary of excitation on tectum initially excites NI, but soon after, NI stops firing because the boundary of inhibition exceeds the boundary of excitation. The parameters of this model could be adjusted for the weak directional selectivity of teleost NI so that nonexpanding, lateral motion is also effective. What is not easily explained is the weak differentiation in the response to the expanding and contracting vertical lines (Fig. 8). Clearly more remains to be done on the effects of the configuration of moving stimuli.

Conclusions

The teleost NI responds to two main aspects of visual stimulation: novelty and motion. Both are seen in play during the approach of an object. First, there is a transient increase in activity at the start of movement and then, as the object nears the animal, a subsequent increase in activity, which, on average, appears to signal proximity of the object. One important question raised is how the tectal-NI circuitry achieves an essentially linear representation of proximity largely independent of speed and object size.

The spatially integrating nature of NI, although belying its topographic connections with tectum, is responsible for a general and synchronous activation of the ipsilateral tectum, particularly in the deeper layers (Northmore & Gallagher, 2003). Although the

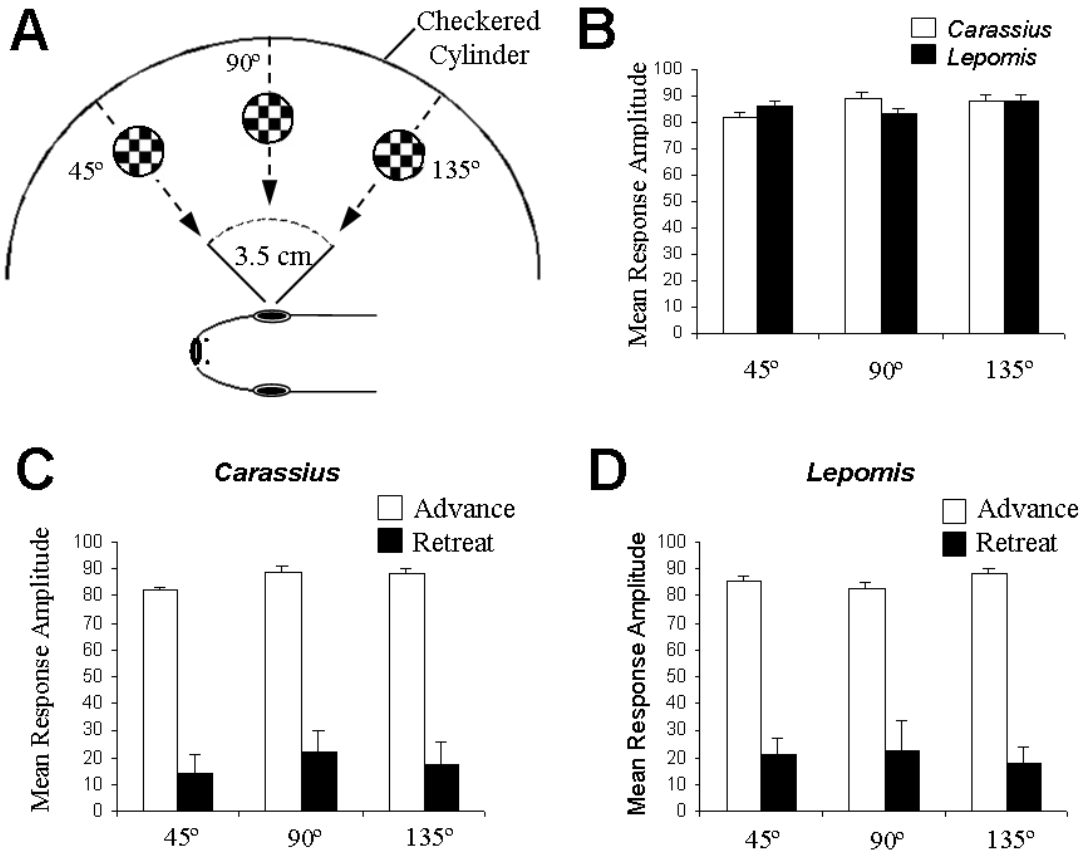

Fig. 7. NI responses in *Carassius* and *Lepomis* to the advance and retreat of a 3-cm checkered ball. (A) The ball advanced in the horizontal plane approaching from azimuths of 45, 90, and 135 deg. In each case the ball traveled 10 cm at approximately 0.4 m/s, stopping 3.5 cm from the eye when it subtended 46 deg. (B) Bar graph showing the mean integrated activity (±SEM) recorded from contralateral NI of *Carassius* and *Lepomis* in response to the ball's advance. Each mean was compiled from 90 responses from three fish (30 x 3) of each species. Advancing balls evoked similar mean multiunit responses, regardless of approach path ($P > 0.1$). (C & D) Mean integrated extracellular activity (±SEM) recorded from *Carassius* and *Lepomis* NI in response to the ball advancing on a collision course toward or retreating from the contralateral eye. Each mean was compiled from 90 responses from three fish (30×3) of each species and mean responses to advances were all greater than the mean response to retreats along the same axis ($P < 0.01$).

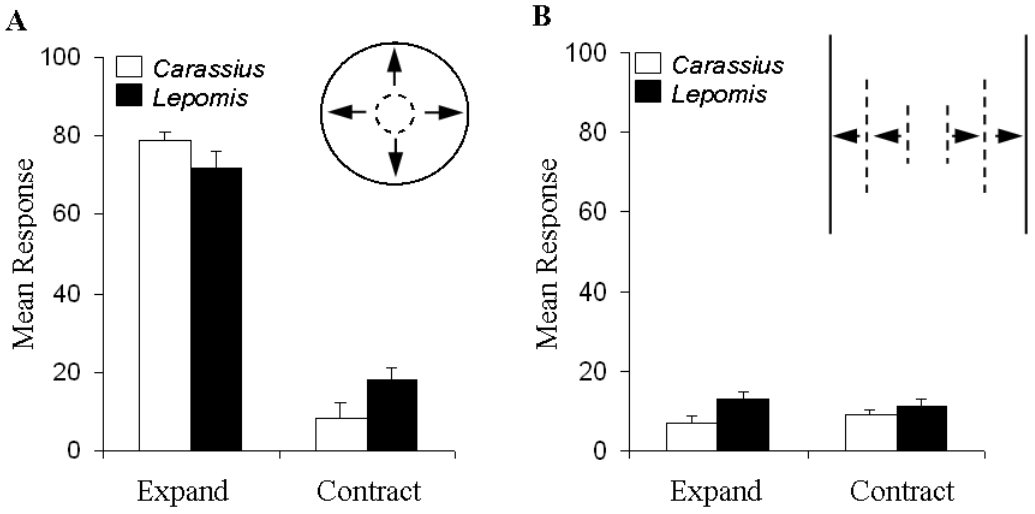

Fig. 8. Mean integrated activity of NI in *Carassius* and *Lepomis* to expanding and contracting two-dimensional images over a range of 15–50 deg of angular subtense at the eye over 0.75 s. (A) Mean activity as a circle expanded and contracted in the contralateral visual field. Mean responses recorded during the ring's expansion were greater than those recorded during the ring's contraction for both species ($P < 0.01$). (B) Mean activity to the expansion and contraction of a pair of vertical lines. Expansion and contraction were equally effective for both species ($P > 0.05$).

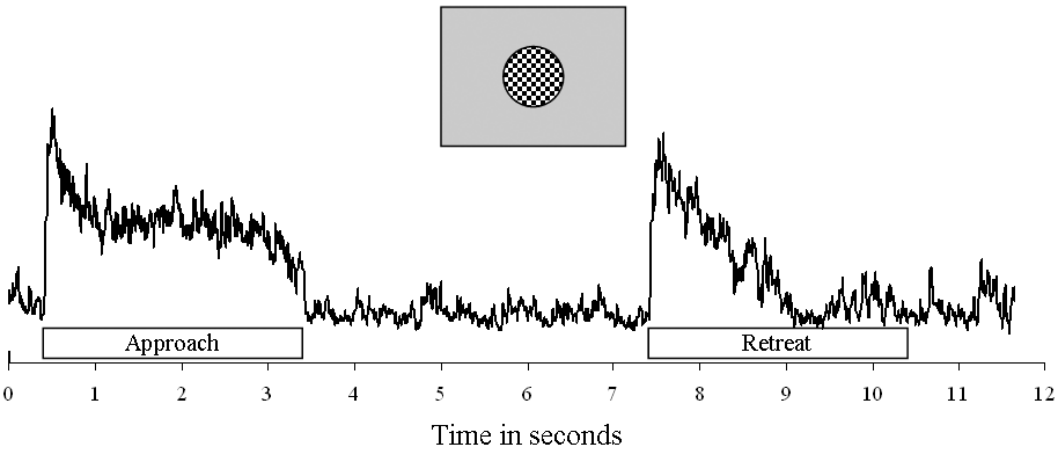

Fig. 9. Integrated responses of NI in *Lepomis* to simulated approach and retreat of a checkerboard pattern viewed through a circular window subtending 20 deg at the eye. In the approach phase, checks expanded so as to simulate an extended checkered surface moving toward the eye perpendicular to the body axis from a distance of 50 to 8 cm at a uniform speed of 15 cm/s; retreat was simulated by a corresponding contraction. Each square of the checkerboard pattern varied in angular subtense from 0.57 deg to 3.6 deg. Simulated motion occurred as indicated by the boxes on the time axis. The activity record is the average of five presentations.

role of synchronous activity in the tectum remains unknown, it is conceivable that NI's signal, representing salient events, is made available for comparison with all current sensory representations across tectum, allowing an appropriate behavioral response to the most important.

Acknowledgments

Greg Poulos provided assistance in the development of computer-generated visual stimuli.

References

Brandis, A. & Saidel, W.M. (2001). Nucleus isthmi: The modulator of binocular vision in fish. *Bulletin of the New Jersey Academy of Sciences* **46**, 22.

Collett, T.S., Udin, S.B. & Finch, D.J. (1987). A possible mechanism for binocular depth judgments in anurans. *Experimental Brain Research* **66**, 35–40.

Cui, H. & Malpeli, J.G. (2003). Activity in the parabigeminal nucleus during eye movements directed at moving and stationary targets. *Journal of Neurophysiology* **89**, 3128–3142.

Dunn-Meynell, A.A. & Sharma, S.C. (1984). Changes in the topographically organized connections between the nucleus isthmi and the optic tectum after partial tectal ablation in adult goldfish. *Journal of Comparative Neurology* **227**, 497–510.

Glasser, S. & Ingle, D. (1978). The nucleus isthmus as a relay station in the ipsilateral visual projection to the frog's optic tectum. *Brain Research* **159**, 214–218.

Graybiel, A.M. (1978). A satellite system of the superior colliculus: The parabigeminal nucleus and its projections to the superficial collicular layers. *Brain Research* **145**, 365–374.

Grobstein, P. & Cromer, C. (1983). The nucleus isthmi as an intertectal relay for the ipsilateral oculotectal projection in the frog, Rana pipiens. *Journal of Comparative Neurology* **217**, 54–74.

Grobstein, P., Cromer, C., Hollyday, M. & Archer, S.M. (1978). A crossed isthmo-tectal projection in Rana pipiens and its involvement in the ipsilateral visuotectal projection. *Brain Research* **156**, 117–23.

Grover, B.G. & Sharma, S.C. (1981). Organization of extrinsic tectal connections in goldfish (Carassius auratus). *Journal of Comparative Neurology* **196**, 471–488.

Gruberg, E.R. (1983). Recent work on the nucleus isthmi and its niche in the visual system. In *Progress in Nonmammalian Brain Research*, Vol. I, ed. Nistico, G. & Bolis, L., pp. 159–174. Boca Raton, Florida: CRC Press.

Gruberg, E.R. & Udin, S.B. (1978). Topographic projections between the nucleus isthmi and the tectum of the frog Rana pipiens. *Journal of Comparative Neurology* **179**, 487–500.

Gruberg, E.R., Wallace, M.T., Caine, H.S. & Mote, M.I. (1991). Behavioral and physiological consequences of unilateral ablation of the nucleus isthmi in the leopard frog. *Brain, Behavior, and Evolution* **37**, 92–103.

Guthrie, D.M. & Banks, J.R. (1978). The receptive field structure of visual cells from the optic tectum of the freshwater perch (Perca fluviatilis). *Brain Research* **141**, 211–225.

Ito, H., Sakamoto, N. & Takatsuji, K. (1982). Cytoarchitecture, fiber connections, and ultrastructure of nucleus isthmi in a teleost (Navodon modestus) with a special reference to degenerating isthmic afferents from optic tectum and nucleus pretectalis. *Journal of Comparative Neurology* **205**, 299–311.

Judge, S.J. & Rind, F.C. (1997). The locust DCMD, a movement detecting neurone tightly tuned to collision trajectories. *Journal of Experimental Biology* **200**, 2209–2216.

Kawasaki, M. & Aoki, K. (1983). Visual responses recorded from the optic tectum of Japanese dace, Tribolodon halonensis. *Journal of Comparative Physiology A* **152**, 147–153.

King, W.M. & Schmidt, J.T. (1993). Nucleus isthmi in goldfish: In vitro recordings and fiber connections revealed by HRP injections. *Visual Neuroscience* **10**, 419–437.

Meek, J. (1983). Functional anatomy of the tectum mesencephali of the goldfish. An explorative analysis of the functional implications of the laminar structural organization of the tectum. *Brain Research Reviews* **6**, 247–297.

Northmore, D.P.M. (1989). Quantitative electrophysiological studies of regenerating visuotopic maps in goldfish. I. Early recovery of dimming sensitivity in tectum and torus longitudinalis. *Neuroscience* **32**, 739–747.

Northmore, D.P.M. (1991). Visual responses of nucleus isthmi in a teleost fish (*Lepomis macrochirus*). *Vision Research* **31**, 525–535.

Northmore, D.P.M. & Gallagher, S.P. (2003). Functional relationship between nucleus isthmi and tectum in teleosts: Synchrony but no topography. *Visual Neuroscience* **20**, 335–348.

O'Benar, J.D. (1976). Electrophysiology of neural units in goldfish optic tectum. *Brain Research Bulletin* **1**, 529–541.

O'Shea, M. & Williams, J.L.D. (1974). The anatomy and output connection of a locust visual interneurone: The lobular giant movement detector (LGMD) neurone. *Journal of Comparative Physiology* **91**, 257–266.

Pérez-Pérez, M.P., Luque, M.A., Herrero, L., Núñez-Abades, P.A. & Torres, B. (2003). Afferent connectivity to different functional zones of the optic tectum of goldfish. *Visual Neuroscience* **20**, 397–410.

Rind, F.C & Bramwell, D.I. (1996). Neural network based on the input organization of an identified neuron signaling impending collision. *Journal of Neurophysiology* **75**, 967–985.

Rowell, C.H.F., O'Shea, M. & Williams, J.L.D. (1977). The neuronal basis of a sensory analyser, the acridid movement detector system. *Journal of Experimental Biology* **68**, 157–185.

Sakamoto, N., Ito, H. & Udea, S. (1981). Topographic projections between the nucleus isthmi and the optic tectum in a teleost, Navodon modestus. *Brain Research* **224**, 225–234.

Schlotterer, G.R. (1977). Response of the locust descending contralateral movement detector to rapidly approaching and withdrawing visual stimuli. *Canadian Journal of Zoology* **55**, 1372–1376.

Sereno, M.I. & Ulinski, P.S. (1987). Caudal topographic nucleus isthmi and the rostral nontopographic nucleus isthmi in the turtle, Pseudemys scripta. *Journal of Comparative Neurology* **261**, 319–346.

Sun, H. & Frost, B.J. (1998). Computation of different optical variables of looming objects in pigeon nucleus rotundus neurons. *Nature Neuroscience* **1**, 296–303.

Vanegas, H. & Ito, H. (1983). Morphological aspects of the teleostean visual system: A review. *Brain Research Review* **6**, 117–137.

Wang, S.-R. (2003). The nucleus isthmi and dual modulation of the receptive field of tectal neurons in non-mammals. *Brain Research Review* **41**, 13–25.

Wang, Y.C. & Frost, B.J. (1991). Visual response characteristics of neurons in the nucleus isthmi magnocellularis and nucleus isthmi parvocellularis of pigeons. *Experimental Brain Research* **87**, 624–633.

Wiggers, W. & Roth, G. (1991). Anatomy, neurophysiology and functional aspects of the nucleus isthmi in salamanders of the family Plethodontidae. *Journal of Comparative Physiology A* **169**, 165–176.

Williams, B., Hernandez, N. & Vanegas, H. (1983). Electrophysiological analysis of the teleostean nucleus isthmi and its relationship with the optic tectum. *Journal of Comparative Physiology A* **152**, 545–554.

Xue, H.-G., Yamamoto, N., Yoshimoto, M., Yang, C.-Y. & Ito, H. (2001). Fiber connections of the nucleus isthmi in the carp (Cyprinis carpio) and Tilapia (Oreochromis niloticus). *Brain, Behavior, and Evolution* **58**, 185–204.