Tectal Control of Locomotion, Steering, and Eye Movements in Lamprey

Kazuya Saitoh, Ariane Ménard, and Sten Grillner

Department of Neuroscience, Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm Brain Institute, Stockholm, Sweden

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First published February 15, 2007; doi:10.1152/jn.00639.2006. The intrinsic function of the brain stem–spinal cord networks eliciting the locomotor synergy is well described in the lamprey—a vertebrate model system. This study addresses the role of tectum in integrating eye, body orientation, and locomotor movements as in steering and goal-directed behavior. Electrical stimuli were applied to different areas within the optic tectum in head-restrained semi-intact lampreys (n = 40). Motions of the eyes and body were recorded simultaneously (videotaped). Brief pulse trains (<0.5 s) elicited only eye movements, but with longer stimuli (>0.5 s) lateral bending movements of the body (orientation movements) were added, and with even longer stimuli locomotor movements were initiated. Depending on the tectal area stimulated, four characteristic response patterns were observed. In a lateral area conjugate horizontal eye movements combined with lateral bending movements of the body and locomotor movements were elicited, depending on stimulus duration. The amplitude of the eye movement and bending movements was site specific within this region. In a rostromedial area, bilateral downward vertical eye movements occurred. In a caudomedial tectal area, large-amplitude undulatory body movements akin to struggling behavior were elicited, combined with large-amplitude eye movements that were antiphasic to the body movements. The alternating eye movements were not dependent on vestibuloocular reflexes. Finally, in a caudolateral area locomotor movements without eye or bending movements could be elicited. These results show that tectum can provide integrated motor responses of eye, body orientation, and locomotion of the type that would be required in goal-directed locomotion.

INTRODUCTION

Goal-directed locomotion, such as catching a prey, escaping from a predator, or avoiding an obstacle, depends on the execution of several basic motor patterns including eye movements, neck bending, locomotion, and steering. They must be recruited in the appropriate sequence and with the correct timing (Ewert 1997; Grillner 2003). Visuomotor coordination is essential for adapting the locomotor pattern to the environment in both mammals (Georgopoulos and Grillner 1989) and lower vertebrates, such as amphibians (Ingle 1971; Kostyk and Grobstein 1987), fish (Gahtan et al. 2005; New et al. 2001; Pettigrew et al. 2000; Rice and Westneat 2005), and cyclostomes (lamprey) (Deliagina et al. 1993). The object of this study is to explore the neural bases of visuomotor coordination in the lamprey, which is used as an experimental model, in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The optic tectum is a main target of visual inputs from retinal ganglion cells in lower vertebrates such as lamprey (Kennedy and Rubinson 1977; Niewenhuys and Nicholson 1998), goldfish (Meyer 1980; Stuermer and Raymond 1989), and zebrafish (Baier et al. 1996; Stuermer 1988). The laminated structures of the optic tectum in cyclostomes (Iwahori et al. 1996, 1999;) and teleosts (Meek 1983; Sajovic and Levinthal 1982) are similar to those of the mammalian superior colliculus (Warton and Jones 1985). The optic tectum is considered a homologue of the mammalian superior colliculus, which serves as a command center for shifting the eyes to a point of particular interest (Moschovakis et al. 1996; Sparks 2002) and also for orienting the head and body in the same direction (Corneil et al. 2002; Isa and Sasaki 2002).

In fish, microstimulation of the optic tectum can evoke not only eye movements but also motor responses related to steering during ongoing locomotion (Al-Akel et al. 1986; Grillner and Wallén 1984; Herrero et al. 1998). These motor responses are specific to the stimulus site in the optic tectum. Ablation of tectum in zebrafish has less effect on basic optomotor responses and optokinetic responses (Roeser and Baier 2003) than on prey capture requiring a precise interaction with the surrounding visual space (Gahtan et al. 2005). The optic tectum thus appears to transform spatial information from the visual field to motor commands, resulting in appropriate eye and orienting movements of the body.

The role of the superior colliculus in gaze shifting and orienting movements was studied in a variety of mammals (Freedman et al. 1996; Grantyn et al. 1996; Roucoux et al. 1980; Sahibzada et al. 1986; Stein et al. 1976; Stryker and Schiller 1975). It is organized as an efferent motor map, in which site-specific saccadic eye movements can be elicited in different directions and with different amplitudes (Robinson 1972). The output from the superior colliculus is channeled by horizontal and vertical gaze centers in the brain stem to the appropriate motor nuclei (for review, see Moschovakis et al. 1996; Sparks 2002) and, in addition, by tecto–reticulospinal pathways as demonstrated in rodents (Dean et al. 1986) and cats (Munoz et al. 1991; Muto et al. 1996; Olivier et al. 1993; for review, see Isa and Sasaki 2002). The collicular motor map itself is under tonic inhibition from the basal ganglia and a saccadic eye movement can be triggered by a site-specific disinhibition of a microregion within the motor map (Hikosaka et al. 2000).

We will explore whether a similar neural control system including the optic tectum is present in the lamprey, representing the earliest group of vertebrates in a phylogenetic perspective. As a first step in this analysis, we report here the effect of microstimulation of the optic tectum on eye and orienting movements. An experimental semi-intact model has been developed, in which the motor response to tectal stimulation of
both eyes and the head-restrained body can be recorded. Four distinct types of coordinated eye–body orienting responses and also locomotion could be elicited from tectum in a topographical manner. These results were previously presented in abstract form (Saitoh et al. 2004).

METHODS

Experiments were performed on sea lampreys [Petromyzon marinus (transformers); body length 15–18 cm, n = 40]. The experimental procedures were approved by the local ethical committee (Norra Djurförörsöktetsd Nämnden) and were in accordance with The Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1996 revision). During the investigation, every effort was made to minimize animal suffering and to reduce the number of animals used.

Surgical procedures

The procedure for making semi-intact preparations was as follows (Fig. 1A). Animals were anesthetized with 0.02% tricane methane sulfonate (MS-222; Sigma, St. Louis, MO). The skin of the head and the epithelial layer of the cornea were removed to directly observe the motion of the eyes. The brain and brain stem were exposed by removing the cartilage above them and the entire telencephalon was resected to obtain a decerebrate condition. The different oculomotor nerves (oculomotor, trochlear, and abducens nerves) were kept intact. In most animals, both optic nerves were cut, but sometimes they were kept intact. The head was pinned down in a silicone elastomer (Sylgard; Dow Corning, Midland, MI) lined cooling chamber (35 cm long, 20 cm wide, and 3 cm deep). To improve the stabilization of the head, the soft tissue on its ventral side was removed. The chamber was continuously perfused with cold artificial saline (8–10°C). The saline contained (in mM): 138 NaCl, 2.1 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 4 glucose, and 2 HEPES and was bubbled with O₂; the pH was adjusted to 7.4. The actual experiment with tectum stimulation and the recording of motor behavior started at least 2 h after surgery when the animal had fully recovered from anesthesia. A sign of recovery was that vigorous eye movements were evoked in response to light touch applied to the vestibular organ through a cartilage deficit of otic bulb. This response is usually inhibited for about 2 h after anesthesia.
Stimulation of the optic tectum

Tungsten concentric, bipolar electrodes SNEX-100 (maximum diameter of exposed center contact in 100-μm cross section, impedance 0.7–1.5 Ω, epoxide insulation; Clark Electromedical Instruments, Pangbourne, UK) were used for electrical stimulation of different sites in the optic tectum. The electrical stimulation (cathodal square-wave pulses) was applied with an isolated pulse stimulator Model 2100 (A-M Systems, Carlsborg, WA), and consisted of a train of pulses (frequency: 5–50 Hz; pulse width: 1–2 ms) with a variable duration (0.1–10 s) and an intensity between 5 and 50 μA. Time intervals between two periods of stimulation were ≥5 min. The tip of electrode was placed on the surface of tectum in each stimulation.

Recording of the motor behaviors and kinematic analysis

Motor behaviors were videotaped with two synchronized digital video cameras (Canon MV600i and Panasonic NV-GS 11, Tokyo, Japan). One video camera was mounted on the microscope (Wild M5A, Heerbrugg, Switzerland) to record the motion of the eyes and the other above the lamprey for recording the body shape and locomotor patterns. The video images were captured by the workstation (Dell Precision 470) with DV format (resolution 720 × 576 pixels, 25 frames/s). Each video frame was analyzed using a computer-aided design (CAD) software (M7, share software) frame by frame. The horizontal motion of eyes was evaluated by measuring the angle of the visual axis with reference to the initial position (denoted by broken arrows; Fig. 1Ba). With forward movement, the value of the angle was denoted as positive and for backward motion, negative (Fig. 1Bb). To evaluate the vertical component of eye movement (downward shifts in most cases), the motion of the eye was sometimes videotaped from the lateral side (Fig. 1Ca). When the length of the vertical axis of the pupil changed from α (maximum) to β (Fig. 1C), the angle of vertical eye movement (θ) was given as follows (Fig. 1Cb)

\[ \theta = \arccos \left( \frac{b}{a} \right) \]

We also evaluated the motion of the body, including neck bending and locomotion, from video images. Here we define the “neck” of the lamprey as the rostral part of body from the level of the first gill-port hole to the seventh gill-port hole (Fig. 1Aa). The branchial muscles of lamprey are innervated by the facial (first gill), glossopharyngal (first and second gills), and vagal nerves (second to seventh gills) (Guimond et al. 2003). This innervation pattern of branchial muscles is very similar to the innervation pattern of the human “neck” muscles that originate from the branchial arches, although the accessory nerves and thus the trapezius muscles are missing in lamprey (for review, see Kuratani et al. 2002). Three angles—α, β, and γ—were defined and measured in each frame. First, we set the origin (O) in the center of the ventriculus mesencephali in the initial video frame (Fig. 1Da). The next three points along the body axis (OX) were defined in the initial video frame (Fig. 1Da). The point aligned with the seventh gill-port hole was defined as P2, halfway between the ventriculus mesencephali (O) and P3, as shown in Fig. 1Db, when the neck bends in a horizontal plane. P1, P2, and P3, shift along concentric arches, the radii of which are OP1, OP2, and OP3, respectively (drawn by dotted arches in Fig. 1D). The angle between line OP and line OP1 was defined as α, the angle between line OP and line OP2 was defined as β, and the angle between line P1P2 and line P1P3 was defined as γ (Fig. 1D, b and c). When the body position shifted contraversive to the stimulus side, the values of these angles were denoted as positive and as negative in the opposite direction (Fig. 1Dc). These three angles indicate the curvatures of upper, middle, and lower neck of the lamprey, respectively. In eel-like fish, the amplitude of the lateral displacement during swimming increases monotonically from head to tail (Grillner and Kashin 1976). We defined “swimming” as repetitive undulatory movements with increasing amplitude of lateral displacement along the body (Fig. 1Ea). The value of each angle oscillates during swimming (Fig. 1Eb). The correlation between α and γ shows high linearity and these two values maintain a phase lag of around 180° during swimming (Fig. 1E, b and c).

Tectal map of effective sites for eliciting coordinated behaviors

To make a tectal map of effective sites for eliciting motor responses (Figs. 7–11), each position of the electrode tip was transferred to a relative position in an x-y coordinate system. In this coordinate system, the origin was put at the caudal left border of the tectum. The position of the right border of tectum was defined as 100% in the x-axis and the caudal end of the posterior commissure as 100% in the y-axis.

Data exploration

The distance-weighted least-squares smoother method was applied for data fitting of scatterplots in Figs. 2 and 4 using SYSTAT software (version 10, Chicago, IL).

RESULTS

Horizontal eye movements induced by electrical stimulation of the optic tectum

Electrical stimulation of different areas within the optic tectum induced horizontal eye movements. Brief stimuli (0.1–0.5 s) induced only eye movements, in contrast to longer stimuli (see following text). Figure 2A shows the amplitude (length of arrows) and direction (upward arrow: forward eye shift; downward arrow: backward eye shift) of horizontal eye movements (see Fig. 1B) elicited in response to stimulation (0.5-s, 40-Hz, 1-ms pulse, 40 μA) of different regions in the left optic tectum in one animal. Each amplitude value is the average of at least three trials in the same stimulus site. The left eye (blue arrow) moved forward and the right eye (red arrow) backward by stimulation of the lateral part of the left optic tectum (Fig. 2A). The direction of eye movement was site specific and never changed between trials. The movement amplitude of both eyes decreased from lateral and caudal locations to the medial part of the optic tectum. At more medial sites the eyes could instead move in the opposite direction (Fig. 2A). Black dots indicate sites where a brief stimulation induced no eye movement. Later we document the effects of different stimulus parameters on the characteristics of the horizontal eye movements.

SPATIAL CHARACTERISTICS OF THE EYE MOVEMENT. Amplitude. Figure 2B shows the peak amplitude of the eye movements obtained with electrical stimuli (40 Hz, 2 ms, 25 μA) of different train duration, applied to either the right or the left rostral/lateral part of tectum in two mirror sites (green symbols in subsets). The peak amplitude for the left (blue) and right (red) eye is represented for two sites at each side of tectum. With a train duration of >0.05 s (three pulses) eye movements were elicited and they increased in amplitude as the stimulus train duration was increased to reach a maximum value at about 0.7 s (Fig. 2B).

In Fig. 2C the effect of changing the stimulus strength is illustrated, while holding the stimulus train duration constant (0.3 s, 40 Hz, and 2 ms) at three different mirror sites in the same animal (Fig. 2C, left and right diagrams). The threshold
for eliciting eye movements was about 20 μA and the peak amplitude increased with stimulus strength up to about 50 μA. With higher current intensities (60–100 μA) the amplitude of the horizontal eye movement remained constant or was somewhat reduced. Stimulation of the mirror sites induced eye movements in the opposite direction, following a similar duration–peak amplitude or current–peak amplitude relation.

**Direction.** The direction of the horizontal eye movement in each site did not change regardless of stimulus current intensities (Fig. 2C) except for the caudomedial part of tectum (Fig. 2D), in which the horizontal eye movements changed direction with increasing current intensity (Fig. 2D). With 10 to 40 μA, the eye ipsilateral to the stimulus site moved backward and the contralateral eye moved forward (n = 2), whereas the direction...
was reversed without drastic changes of peak amplitude with higher currents (50–80 μA) (Fig. 2D).

Oculomotor map. To obtain an oculomotor map of the optic tectum, we determined the optimal stimulus train duration and current intensity that elicited the maximum amplitude of antiphasic horizontal eye movements. A train duration of 0.7 s (40 Hz, a pulse duration of 1 ms) was used for all animals and a current intensity between 35 and 45 μA. We investigated the amplitude and direction of the evoked horizontal eye movements. In each animal (n = 4), electrical stimuli were applied to 18 areas of the superficial layer of tectum (Fig. 3A, a–d). In Fig. 3A, the actual amplitude and direction of the eye movement in each animal are shown on both the ipsi- and contralateral sides. The effective sites are indicated by filled circles on the tectal maps (the center of each circle indicates the location of the electrode tip). In red circles the eye moved forward and in blue circles, backward. The amplitude is indicated by the diameter of each circle.

The amplitude of the ipsi- and contralateral eye movements elicited from different parts of tectum are compared in Fig. 3B. The response patterns from the lateral (red; see inset) and medial (green) parts are shown (Fig. 3Ba). It is clear that the direction is opposite for both eyes in the medial and the lateral parts, respectively. Furthermore the amplitude of the eye movements on the two sides is correlated. Figure 3Bb compares instead rostral (yellow; see inset) and caudal (blue) stimulation points. A similar pattern with respect to the peak amplitude of both eyes is apparent, although the ipsilateral eye on average tends to have a slightly larger peak amplitude (P < 0.05) than that of the contralateral eye (n = 49 trials from the same four animals; Fig. 3, B and D).

Figure 3C shows a summary of the normalized amplitude and direction of tectal-induced eye movements obtained from these four animals for the ipsi- and contralateral eyes. The peak amplitudes of the contraversive eye movements increased in the areas colored from yellow to red in the map of the ipsilateral eye (Fig. 3C, Ipsi. eye) and in the corresponding areas in the map of the contralateral eye (blue in Fig. 3C, Contra. eye). Later, we will refer to eye movements, in which the eye ipsilateral to the stimulus site moves forward and the eye on the contralateral side backward, as contraversive and eye movements in the opposite direction as ipsiversive.

TEMPORAL CHARACTERISTICS OF THE EYE MOVEMENT. Latency, rise time, and decay time. Next we tested the effect of current intensity on the temporal properties of the horizontal eye movements in terms of latency, rise time, and decay time (Fig. 4A). The latency from the onset of stimulation to the onset of eye movement (a in schematic * of Fig. 4A) decreased to around 0.04 s as the current intensity was increased (Fig. 4Aa). On the other hand, the time from the onset of eye movement to peak amplitude (the rise time, b in schematic * of Fig. 4A) remained between 0.16 and 0.20 s with increasing current intensity (Fig. 4Ab; see legend), although the amplitude of the movements increased. The decay time, in which the eyes move back to the initial position (c in schematic * of Fig. 4A), increased within the range between 20 and 40 μA and then remained constant (Fig. 4Ac).

Peak angular velocity. The angular velocity of horizontal eye movement was estimated by measuring the absolute angular difference of visual axis between successive video frames (|Δθ/Δt| | Δt = 0.04 s, histogram in Fig. 4Ba) when stimuli (20 Hz, 2 ms, 0.3 s, and 25 μA) were applied to the left tectum. The peak angular velocity was 65.5°/s (Fig. 4Ba) and occurred in the middle of the first initial eye movements (solid line in Fig. 4Ba). The relationship between the peak angular velocity and stimulus frequencies is represented in Fig. 4Bb for a stimulus point in the rostralateral part of tectum, which showed a linear increase with stimulus frequency in the range from 5 to 40 Hz (black dots in Fig. 4Bb). With higher stimulus frequency of 40–50 Hz (1–2 ms, 0.3–0.5 s, 25–50 μA), the peak angular velocity reached ≥250°/s (62.5–258°/s, 14 trials from eight sites in five animals) in the rostralateral part of tectum (Fig. 4C). The peak angular velocity was positively correlated with the peak amplitude of eye movements (Fig. 4C).

Stimulus current threshold for eliciting eye movement, neck/trunk bending, and swimming

We investigated stimulus current thresholds for eliciting not only eye movements, but also neck/trunk bending and swimming. Electrical stimuli (40 Hz, pulse duration of 1 ms) with various current intensities were applied to 18 areas of the left tectum (Fig. 5). The train duration was either 0.2 s (Fig. 5A) or 2 s (Fig. 5B). To determine the threshold, the current intensity was increased gradually from 5 μA with 2.5-μA steps (color coded) until the different motor behaviors were elicited. When no behavior was observed at 50 μA in a given area, we considered the electrical stimulation to have “no effect.” When the train duration was 0.2 s, the threshold current for neck/trunk bending was higher than that of eye movements in most areas. Swimming was elicited from only two of 18 sites. When the train duration was changed to 2 s, swimming was induced in 12 of 18 areas (Fig. 5B). The current threshold for swimming was significantly higher than that of other motor behaviors. In addition, both neck/trunk bending and swimming showed higher threshold currents when train duration was 0.2 s, compared with those when the train duration was 2 s. Thus in general the shorter train durations had higher intensity thresholds, although there is a hierarchy among the movements between the eye, neck/trunk, and swimming with respect to stimulus threshold.

Behavioral change related to stimulus train duration

Figure 6 shows the effect of stimulus train duration on the type of motor pattern elicited in different areas of tectum (40 Hz, a pulse width of 1 ms, 30 μA). The stimuli were applied in 18 sites of the left tectum and the stimulus duration was progressively increased from 0.1 to 3 s in each area (from left to right in Fig. 6). Very brief stimuli with durations of 0.1 to 0.3 s induced only eye movements (dot pattern panels). When the duration was increased to 0.5 s, neck/trunk bending followed the eye movement in some locations (gray panels). Stimuli with 0.7 to 0.9 s also induced swimming (black panels). Very long stimuli with a duration of 3 s elicited swimming in most areas where effects were observed. Eye movements were observed in 14 of 18 stimulus sites and, in 11 of these, the eye movements were followed by neck/trunk bending and swimming on increasing stimulus duration. The order of the behavioral change from eye movement, neck/trunk bending, to swimming was well maintained in all animals in
FIG. 3.  Tectal map of amplitude and direction of horizontal eye movements.  

A: effective sites for eliciting horizontal eye movements of the ipsilateral (Ipsi.) and contralateral eye (Contra.) for 4 animals (a–d). Each dorsal view of optic tectum (a–d) was traced from video images of each animal. Actual peak amplitude is shown by the diameter of each circle. Color of each circle indicates the direction of the motion of the eye (red, forward; blue, backward). Each black bar indicates the scale of 0.2 mm.  

B: scatterplots of peak amplitudes of contralateral eye against those of ipsilateral eye. Plot distributions were compared (a) between eye movements elicited from lateral part of tectum (red dots) and medial part of tectum (green triangles), and (b) between eye movements elicited from rostral part of tectum (yellow inverse triangles) and caudal part of tectum (blue squares).  

C: contour maps of horizontal components of the motion of both eyes. Color scale indicates the normalized and averaged amplitudes obtained from 4 animals. Color scale ranged in 10 steps from the initial position (green) to the position with the largest amplitude of forward eye movements (red) and 10 further steps for backward movements (from green to blue). Each circle represents one stimulus site within each tectal area.  

D: comparison of absolute peak amplitudes between ipsilateral eyes and contralateral eyes using paired t-test. Data were obtained from 49 trials in 4 animals shown in A (a–d).
which the effects of stimulus duration on the pattern of motor behavior were investigated (n = 6).

Oculomotor and locomotor patterns elicited from stimulation of the optic tectum

The motor patterns evoked by stimulation of tectum were classified into four types based on the oculomotor, trunk, and locomotor patterns: type I (Fig. 7), type II (Fig. 8), type III (Fig. 9), and type IV (Fig. 10).

**TYPE I MOTOR PATTERN: HORIZONTAL ORIENTATION MOVEMENTS AND LOCOMOTION (SUPPLEMENTAL MOVIE 1).** Both eyes showed a predominant horizontal single excursion in a particular direction in antiphase (denoted by open arrowheads in Fig. 7, A and B). The direction of the induced eye movement was contraversive (ipsilateral eye forward and contralateral eye backward) to the stimulus site in 62 of 66 effective sites (94%; black bars in Fig. 7C). Figure 7A shows the contraversive movements of the eyes resulting from a brief stimulation (0.7 s). After cessation of the stimulation, alternating body movements appeared, starting caudally and engaging progressively more rostral regions (broken line with arrowhead in Fig. 7A). When the duration of the stimulus was increased to 10 s (Fig. 7B), contraversive eye movements occurred but the eye position shifted gradually back to the initial position during stimulation, whereas swimming movements began and the overall neck angle changed to some degree (gray broken line in Fig. 7B, a, b). In roughly 80% of these sites (49/62; Fig. 7C) the neck/trunk was also bent in a contraversive direction, which is a form of orienting response. The peak amplitude and direction of the horizontal eye movement were correlated to the peak amplitude of the neck bending (a) and right and left eye movements were 0.56 and 0.49, respectively. Neck bending was missing in 17% of the effective sites (11/66; Fig. 7C) and eye movement followed by swimming alone was also observed. In four sites (four of 66) ipsiversive eye movements resulted and in one of these the neck/trunk movements were also ipsiversive. Type I motor patterns could be elicited by stimulation of the large predominantly lateral area of tectum as indicated in Fig. 7F (66 effective sites, 18 animals).

**TYPE II MOTOR PATTERN: RHYTHMIC EYE AND TRUNK MOVEMENTS (SUPPLEMENTAL MOVIE 2).** The type II motor pattern is fundamentally different from type I in that the two eyes exhibit large rhythmic movements in antiphase as most clearly seen in Fig. 4B: effects of stimulus current intensity on latency (a), rise time (b), and decay time (c) of elicited horizontal eye movements (schematic, *). Stimuli with various current intensities (10−80 μA, 40 Hz, 2 ms, 0.3 s) were applied to the tectum. A: angular velocity in each video frame (|Δθ/Δt|, Δt = 0.04 s; gray bar chart) of horizontal eye movements. Stimulation with 20 Hz, 2 ms, 0.3 s, 25 μA was applied to the tectum. Angle of visual axis during horizontal eye movement (θ; black line chart) was superimposed. Video frame “1” was defined as the first frame in which the horizontal eye movement was observed. b: relationship between peak angular velocity of horizontal eye movement and stimulus frequency for a rostrolateral stimulus site (filled circle; 1 ms, 0.5 s, 50 μA). C: peak angular velocity as a function of peak amplitude of horizontal eye movement. Stimuli with 40 (filled circles) or 50 Hz (open squares) (1–2 ms, 0.3−0.5 s, 25−50 μA) were applied to the lateral part of tectum.

![Fig. 5. Tectal map of threshold current intensities for eliciting eye movements, neck/trunk bending, and swimming. A: electrical stimuli (40 Hz, 1 ms) with train duration of 0.2 s (a) and 2 s (b) were applied to 18 areas of the left tectum in one animal. Current intensities were varied from 5 to 50 μA with 2.5-μA steps.](image-url)
8B. The eye movements are coordinated with large-amplitude neck/trunk movements (Fig. 8B, filled arrowheads and arrows) that are also shown as superimposed video frames from one cycle (Fig. 8D). After the termination of the stimulation period ordinary locomotor movements are present (Fig. 8E). Even with a short period of stimulation (0.5 s; Fig. 8A) antiphase eye and neck movements were induced (filled arrowheads and arrows in Fig. 8A). The movement pattern of the eyes and neck showed a high degree of synchrony (Fig. 8C). The amplitude of the neck/trunk movements is clearly much larger than that of swimming (compare Fig. 8, D and E) and the cycle duration slower (3.1 and 1.3 s, respectively). The cycle durations during tectum-induced neck/trunk movements and during poststimulus swimming were evaluated from autocorrelograms (Fig. 8, F and G) obtained from α plots during the stimulation [hatched square “F” (6 s) in Fig. 8B] and during the poststimulus swimming [hatched square “G” (6 s) in Fig. 8B]. In contrast to the type I response, the swimming response in type II always occurred after the stimulation was finished (Fig. 8B). Type II responses were elicited from 36 sites in a circumscribed mediocaudal region (filled triangles in Fig. 8H, 27 animals), clearly separate from the tectal area giving rise to the type I response (Fig. 7E).

TYPE III MOTOR PATTERN: DOWNWARD EYE MOVEMENTS (SUPPLEMENTAL MOVIE 3). In type III patterns, both eyes moved downward (Fig. 9A, gray arrows in Fig. 9C) directly after the onset of stimulation (latency <80 ms), and then gradually upward until the initial position was resumed (Fig. 9A). In some cases both eyes in addition moved forward (black arrows in Fig. 9C; convergence) in advance of the downward motion of the eyes. An exact evaluation of the horizontal component could not be obtained while the eyes moved vertically under our recording conditions (thus the shaded area in Fig. 9, B and C; see

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**FIG. 6.** Representative example of behavioral change related to the train duration (from 0.1 to 3 s). Intensity (50 μA), frequency (40 Hz), and pulse width (1 ms) were not changed. Each panel indicates the motor pattern induced from each tectal stimulus area.

**FIG. 7.** Type I motor pattern. A and B: oculomotor and locomotor patterns in type I induced by electrical stimuli with short duration (A; 0.7 s, 40 Hz, 1 ms, 50 μA) and long duration (B; 10 s, 40 Hz, 2 ms, 27.5 μA). Topmost trace in each diagram indicates motion of the right eye (black trace) and left eye (gray trace). Bottom 3 traces indicate the locomotor pattern represented at 3 different levels along the body (angles: α, β, γ; see Fig. 1). Eyes move in opposite direction (open arrowheads in A and B). Neck bending follows the eye movement (arrows in A). Gray broken lines in B indicate the change of overall neck angle. C: summary of the direction of eye movements and neck bending elicited from the stimuli of 66 optimal sites in 18 animals. Black bars indicate stimulation sites that produced contraversive (contv.) eye movements and are subdivided with respect to the accompanying neck movements in contraversive (contv., n = 49), ipsiversive (ipsiv.; n = 4), and no neck movements (--; n = 9). Corresponding data for ipsiversive movements are shown with open bars. D: correlation between horizontal eye movements and neck bending (α). Twenty-three stimuli were applied to 7 effective sites (gray rectangles) for eliciting both horizontal eye movements and neck bending in one animal (0.9 s, 40 Hz, 1 ms, 50 μA). Correlation coefficients (r) for eye (forward and backward) and neck movements are indicated. E: optimal sites for eliciting type I motor pattern (filled circles).
METHODS) but slow horizontal eye movements did occur at the end of the vertical eye movements (filled arrowheads in Fig. 9, B and C; see also white arrow in Fig. 9C). The neck/trunk bending was prominent (Fig. 9, D and E). Figure 9, D and E shows superimpositions of five video images during a C-shaped body bending (rectangle in Fig. 9B) and an S-shaped body bending (rectangle in Fig. 9C), respectively. Following the initial C or S start, swimming was initiated during and/or after the stimulation (Fig. 9, B and C). Type III responses were elicited in eight sites obtained from six animals. These sites were restricted to a rostromedial area in tectum (filled squares in Fig. 9F).

**TYPE IV MOTOR PATTERN: FORWARD LOCOMOTION (SUPPLEMENTAL MOVIE 4).** In the type IV motor pattern, swimming could be elicited during the stimulation (Fig. 10B) without initial eye movements (Fig. 10, A and B). Unlike the type I, II, and III motor patterns, eye movements alone were not elicited by stimuli applied to the effective sites, even when the stimulus duration or intensity was decreased below the threshold for evoking swimming. Sometimes small-amplitude movements of both eyes (<5°; open arrowheads in Fig. 10B) in antiphase were observed late during the swimming episode. These small eye movements were in phase with the fast swimming (Fig. 10D). Type IV responses were elicited only from a caudal region. Eighteen effective sites were obtained from eight animals (filled triangles in Fig. 10C).

**EFFECTIVE STIMULATION SITES FOR TYPE I–IV RESPONSES: A COMPARISON.** Figure 11 shows the tectal areas in which the four different motor patterns were elicited, based on 128 effective sites from 40 animals. There is only a limited overlap between the different areas and it thus appears that each area is specialized for the different motor tasks represented by the type I–IV patterns. Within each area there may be a further specialization, as in the type I area, in which eye movements with different amplitudes can be generated in different parts of this tectal region (Figs. 2A and 3).

**Eye movements during turning, initiation, and termination of swimming**

The eye movements occurring during spontaneous episodes of locomotor activity were also considered. Although no or very small eye movements occurred during locomotion at a neutral angle (open arrowheads in Fig. 12, A and D), the situation changed during turning (Fig. 12A) when clear eye movements occurred (right eye, black trace) synchronized with the locomotor pattern (α, gray trace). Figure 12B shows the superimposition of four video images during turning, when the position (angle) of the body changed (arrow in Fig. 12B). The angle of the eye followed the body angle (shaded area in Fig. 12A). These eye movements disappear as the neutral angle is restored (filled arrowheads in Fig. 12A). As shown in the shaded area of each diagram in Fig. 12, clear-cut eye movements are noticeable when the swimming movements were modified as during turning or termination. During initiation of locomotion, phasic eye movements also occurred (Fig. 12C) but the coordination with locomotion was less clear. These different types of eye movements were observed in all 40 animals investigated. This response pattern was common, irrespective of whether they occurred during spontaneous episodes of swimming or were evoked by tectal stimuli.

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Repetitive eye movements elicited from tectum in the type II motor pattern: neuronal mechanisms

The eye movements observed in type II show very large oscillations synchronized to a left–right bending of the neck (Fig. 8B). To explore the origin of these rhythmic eye movements four possibilities were considered. The coordination between eye and locomotor movements might thus be elicited by 1) vestibular inputs (vestibuloocular reflexes), 2) inputs from mechanoreceptors in the neck region, 3) feedback from activity of the locomotor CPGs at brain stem and spinal cord level (Dubuc and Grillner 1989; Vinay and Grillner 1993), or 4) that they are generated by networks within the brain stem. To test the first three possibilities, the following lesion experiments were performed (Fig. 13B): 1) a bilateral labyrinthectomy to abolish the vestibular input (L), 2) a resection of ventral and dorsal roots 1–15, in addition to pinning down of the preparation at the caudal level of seventh gill to restrict all neck movement (R), 3) spinalization at the caudal level of the obex (S), and 4) different combinations of these lesions (R/L, L/H11001, R/L/H11001).

Fig. 9. Type III motor pattern, characterized by vertical eye movements. A: vertical eye movements elicited by stimulation applied to the rostromedial part of the left tectum (3 s, 50 Hz, 2 ms, 50 μA), as indicated by the angle θ (see METHODS). B and C: horizontal eye movements and trunk and locomotor movements shown to be potentially induced by electrical stimuli with both short duration (B; 0.5 s, 40 Hz, 1 ms, 50 μA) and long duration (C; 10 s, 30 Hz, 2 ms, 35 μA). As shown in gray arrows in C, both eyes moved downward (denoted by shaded areas in B and C; the horizontal component could not be resolved during these conditions). Filled arrowheads in B and C indicate the relatively slow horizontal components following the vertical eye movements as seen in C (white arrow). Changing body shape during the periods indicated by the rectangles in B and C is shown in D and E as successive video frames. D: C-shaped body configuration. E: S-shaped body bending. F: type III motor pattern could be elicited from a restricted rostromedial area (filled squares).

Fig. 10. Type IV motor pattern. A and B: oculomotor and locomotor patterns (α, β, γ) in type IV induced by electrical stimuli with short duration (A; 0.9 s, 20 Hz, 2 ms, 30 μA) and long duration (B; 10 s, 10 Hz, 2 ms, 35 μA). Open arrowheads indicate repetitive movement of both eyes in antiphase with relatively small amplitude (see RESULTS for further details). C: optimal sites for eliciting type IV motor pattern (filled reverse triangles). D: enlarged detail showing the correlation between the small eye movements [right (Rt) eye] and the locomotor movements (β) from B. Calibrations are indicated on the ordinates to the left and right.
The rhythmic eye movements evoked by tectal stimulation (type II region) remained after resection of dorsal and ventral roots (Fig. 13A, R) and also when combined with bilateral labyrinthectomy (Fig. 13A, R/H11001L). This clearly indicates that vestibular or neck reflexes are not required. If in addition a transection at obex was performed, removing all inputs from the spinal cord (Fig. 13A, R/H11001L/H11001S), the large amplitude rhythmic eye movements could still be evoked by tectal stimulation. Table 1 summarizes these results obtained from 16 animals. Taken together, they indicate that the rhythmic eye movements are not dependent on either vestibular inputs or inputs from the spinal cord. Furthermore, swimming elicited after stimulation (denoted by open arrowheads in Fig. 13A) is not attributed to the left–right neck oscillation because swimming was also observed in the neck-restrained animal (Table 1, R, R + L). As apparent from Table 1, the oscillations could be reliably elicited during all the different lesions and must depend on central brain stem networks and must thus be attributed to central networks in the brain stem.

**DISCUSSION**

A major outcome of the present study is that stimulation of the lamprey optic tectum induces different types of eye and locomotor movements in a topographical manner (Fig. 11). Our results show that already in cyclostomes (lamprey)—a vertebrate group that diverged from the main vertebrate line 450 million years ago—tectum has a similar role to that in fish and other vertebrates (see Akert 1949; Lunenburger et al. 2001; also see INTRODUCTION). The basic features of this neural organization thus appear to be conserved since early on in vertebrate phylogeny. We report here that, depending on location and stimulus duration within tectum, different types of eye, trunk, and locomotor movements will occur (types I–IV).

**Estimate of stimulated area**

It is difficult to evaluate the exact area that is activated by electrical stimulation under our experimental condition. However, we could deduce the effectively excited area from electrical stimulation by investigating threshold current intensities for eliciting motor patterns (Fig. 5) to some degree. For example, neck/trunk movements were elicited with a current intensity of 5 μA (train duration of 0.2 s, 40 Hz, 1 ms) in a central region of tectum (red color-coded panel in “Neck and Trunk” in Fig. 5A), although no motor behavior was elicited 200 μm away with a current intensity of even 50 μA and, consequently, less at lower current strength (see METHODS).
The input channels to the lamprey tectum

A prominent visual input to tectum is well established in all vertebrates, including the lamprey (Nieuwenhuys and Nichol-son 1998). The visual projections terminate mainly in the superficial layers of the contralateral tectum in lamprey (Kennedy and Rubinson 1977; Rio et al. 1996) as in teleosts (for review, see Meek 1983). The organization of the retinotectal projections in the latter have been studied in several species (Schwassman and Kruger 1965) and a retinotopic organization similar to that of the mammalian superior colliculus has been demonstrated. The lamprey tectum would therefore also appear to be homologous to the mammalian superior colliculus. The collicular afferents from the basal ganglia are organized in a similar way in different tetrapods, from mammals to amphibians (Marin et al. 1998). The superior colliculus receives a powerful GABAergic control from the basal ganglia (for reviews, see Grillner et al. 2005; Hikosaka et al. 2006). The lamprey tectum also receives prominent

### TABLE 1. Results of lesion experiments

<table>
<thead>
<tr>
<th>Input</th>
<th>Condition</th>
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<tbody>
<tr>
<td>Vestibular</td>
<td>No Lesion</td>
</tr>
<tr>
<td>Stretch receptors of neck</td>
<td>+</td>
</tr>
<tr>
<td>Locomotor CPG</td>
<td>+</td>
</tr>
<tr>
<td>Rhythmic eye movement</td>
<td>12/12</td>
</tr>
<tr>
<td>Left–right neck bending</td>
<td>12/12</td>
</tr>
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</table>

Seven conditions are as follows: without lesions, with labyrinthectomy (L), neck receptor inactivation (R), R + L lesion, spinal cord transaction at obex (S), L + S and R + L + S, with respect to the presence of vestibular, neck afferents, and input from the spinal cord locomotor CPGs (+ or −). The bottom three rows indicate all the experiments in which rhythmic eye movements, left–right trunk movements, and locomotor activity occurred. Under all conditions the interventions neither abolished rhythmic eye movements, nor, when applicable, the other types of movements. The numbers in parentheses for each condition indicate the number of animals used for each type of experiment.
The output channels from tectum to eye, neck, and locomotor centers

Little is known about tectal efferents regarding eye movements in lamprey, but eye muscles and their motor nuclei are organized in a similar way to that of other vertebrates (Fritzsch et al. 1990). In goldfish, the output from optic tectum is channeled to the different eye and trunk motor nuclei by a horizontal and a vertical gaze center located in the mesencephalic reticular formation [Ángeles Luque et al. 2005; Fig. 14, blue arrow (1)]. This neural organization is similar to that in mammals (Corneil et al. 2002; Moschovakis et al. 1996) and may apply also to lamprey.

In cats, medial and lateral reticulospinal tracts receive powerful excitatory input from the superior colliculus that contributes to the control of neck premotor and motoneurons [Grantyn and Berthoz 1987; Grantyn et al. 1987; Sasaki et al. 2004; for review, see Peterson 2004; Fig. 14, blue arrow (2)]. Tecto–reticulospinal projections, which are essential for coordinated eye–neck movements in mammals, are also present in teleosts (Fritzsch et al. 2004; Robertson et al. 2006). In mammals, superior colliculus projections to the MLR were previously identified (Graham 1977; Redgrave et al. 1990). Thus tectal efferents to both DLR and MLR might serve to induce goal-directed locomotion. The relative contribution of the tecto–reticulospinal pathway (Fig. 14, blue arrows) and the tecto–MLR/DLR–reticulospinal pathway (Fig. 14, red arrows) remains to be elucidated. The commands to execute the orienting response need to be integrated with eye movements, steering, and locomotion.

Properties of tectum-evoked horizontal eye movements

Under our experimental conditions, the peak amplitude of horizontal eye movements increased with longer train durations (Fig. 2B) and higher stimulus current intensities (Fig. 2C) to reach a maximum after which the amplitude tended to decrease somewhat. Neck/trunk bending could usually be evoked by current intensities lower than those required for the peak amplitude of the eye movements. If the reduction of the eye movement amplitude was related to the onset of the neck–trunk movement, the amplitude could have been higher during part of the 40-ms duration of each frame duration. Then the peak angular velocity could have been underestimated because the peak velocity could have been higher during part of the 40-ms duration of each frame duration. Then the peak angular velocity could have been underestimated in our study. Second, the range of stimulus frequency we usually used (5–50 Hz) is much lower than that used in previous works (400–500 Hz).

Horizontal orienting movements combined with locomotion: type I response

The dominating response with brief tectal stimuli was a fast horizontal eye movement toward the contraversive side: the more lateral and caudal the stimulus applied, the larger the amplitude. With stimuli of the medial tectum, the eyes instead rotated in the ipsiversive direction (Fig. 2A). A similar reversal of direction of tectum-induced eye movements was also described in barn owl (du Lac and Knudsen 1990) and goldfish (Salas et al. 1997) but not in the monkey (Robinson 1972).

With a longer stimulus train of the same intensity, a bending of neck and trunk occurred. With such neck and trunk config-
uration, the lamprey would, if allowed to swim freely, swim in the direction of the gaze—an orienting movement (Fig. 7). After the stimulation, the eye returned to its original position. As shown in Fig. 7A, the velocity of the eye movements is slower in the return phase than in the orienting phase (compare filled arrowheads with open arrowheads). This is in contrast to the slow and rapid phases of the vestibuloocular reflex, in which the eyes return quickly to the initial position (Beck et al. 2004; Tabak et al. 1996). Under our experimental conditions the head is restrained and the vestibuloocular reflex will be less effective; moreover, passive factors such as the elasticity of extraocular muscles may contribute to the return. Amplitudes of eye and body movements were correlated (Fig. 7D). These coordinated eye and neck/trunk movements might be channelled through the same neural structures [Fig. 14, blue arrows, (1) and (2)].

With an even longer stimulus train, everything else being equal, the orienting movements were combined with locomotion in the new direction. Projections from tectum to the brain stem locomotor areas may account for this effect (see above). In the goldfish, tail beats have been reported as a result of tectal stimulation that may be considered as a sign of locomotor activity (Herrero et al. 1998). In monkeys and cats, locomotor responses to collicular stimulation have not been reported, but this may be explained by the constrained experimental conditions (McIlwain 1986; Schiller and Stryker 1972; Stanford et al. 1996). Tectal stimulation (implanted electrodes) in freely moving rats initiates a variety of behaviors including orienting and avoidance according to stimulus sites and intensity (Sahibzada et al. 1986).

Vertical eye movements combined with trunk movements: type III response

Stimulation in the most rostral and medial position in tectum produced vertical downward movements of both eyes, which slowly returned to the original position (Fig. 9A), and a C-shaped neck bending (Fig. 9D). The latter is commonly observed as the first part of escape responses in fish (Budick and O’Malley 2000; Eaton et al. 2001; Herrero et al. 1998). Because the head is restrained in our preparation, we cannot exclude the possibility that this body movement represents a withdrawal-type startle response, an observed in freely moving larval (Currie and Carlsen 1987) and adult lamprey (McClellan and Grillner 1983; Ullén et al. 1995).

The distribution of effective sites for evoking the type III motor pattern is close to the pretectum–tectum border (Fig. 9F). Thus it cannot be ruled out that type III behavior may be elicited by antidromic activation of the pretectum by projection fibers from pretectum to the optic tectum (Robertson et al. 2006).

Locomotor movements without initial eye movements: type IV response

In a circumscribed area in the most caudal and lateral part of tectum, initial eye or trunk movements were not elicited, but instead only well-coordinated locomotor movements. This may possibly correspond to a straight orientation associated with direction-specific locomotor movements.

Rhythmic coordinated trunk and eye movements in antiphase: type II response

Large-amplitude alternating movements of the trunk—distinctly different from ordinary locomotor movements—were induced by stimulation of a mediocaudal tectal region (Fig. 8). These large-amplitude movements were accompanied by large antiphasic rhythmic horizontal eye movements.

The coordinated rhythmic eye and trunk movements were unique to this tectal location. Normally, the large body movements would substantially displace the eyes, and it would seem purposeful to counterrotate the eyes to maintain a visual image on the retina. This function would correspond to that of the vestibuloocular reflex during terrestrial locomotion. Although vestibuloocular reflexes were previously found in teleosts (Beck et al. 2004) and lamprey (Rovainen 1979), they do not play a role here because the rhythmic eye movements (Fig. 13 and Table 1) remained after bilateral labyrinthectomy. Because the coordination is arranged in a similar way during the type II responses, the possibility may be considered that part of the same brain stem circuitry could be used. In mammals, neuronal connections between the superior colliculus and the vestibular nuclei were described (Izawa et al. 1999; Kitama et al. 1995) and reciprocal inhibitory effects between the right and left vestibular nuclei occur through commissural fibers (Carleton and Carpenter 1983). Whether there are tectal efferents to the vestibular (octavomotor) nuclei in lamprey is not yet known.

Neither an improved fixation of the neck region combined with sensory denervation that excluded peripheral neck receptors nor a transection of the spinal cord at the obex removed the rhythmic eye movements. The tectal stimuli are thus able to elicit rhythmic eye movements through brain stem networks that become coordinated with the networks producing the large-amplitude excursions, presumably through an interaction at the brain stem level.

Lateral neck oscillations were observed in type II (Fig. 8B). Photic stimulation to the tail of freely moving lamprey (Deliagina et al. 1995) or tactile stimulation to the tail of lamprey in a maze (Archambault et al. 2001) induces a similar motor pattern. Such stimuli induce lateral rhythmic oscillations (1–2 Hz) of the head, which can be interpreted as searching behavior (Archambault et al. 2001) or struggling movements (Kasicki and Grillner 1986).

Eye movements may be synchronized to locomotor movements during turning

During ongoing locomotion rhythmic eye movements usually do not occur, but during turning clear rhythmic eye movements in phase with the locomotor movements can be observed (Fig. 12A). These eye and neck (a) movements constitute a slow shift in the baseline and repetition of oscillating movements. As shown in Fig. 12A, when the axis of the neck shifted to the right side (arrow in Fig. 12B), the baseline of the right eye shifted in a forward direction. This coordinated motion is well suited to reset the visual axis to the center of the orbit like the rapid phase of vestibular nystagmus. Because the brain stem circuits, not the vestibular system, drive the rapid phase of vestibular nystagmus, this fast eye movement could be evoked successively in response to the gradual shift of the neck axis. The oscillating eye movements observed might be...
generated through a similar neural mechanism that is used during the searching behavior in type II responses, and thus rely on central mechanisms at the brain stem level. One source for this eye–locomotor coordination during turning movements is the input from the spinal locomotor CPG, which is known to rhythmically entrain the activity of reticulospinal and vestibulospinal neurons (Dubuc and Grillner 1989; Vinay and Grillner 1993). This type of coordination would seem purposeful for maintaining a visual image on retina during turning and also under transient conditions as during termination and initiation of locomotion (Fig. 12, C and D).

In conclusion, a stimulation of a microregion within tectum may correspond to a situation, when an “interesting” object has activated retinal ganglion cells in a given location that in turn topographically activates a local spot in tectum, leading to an orienting movement (eye, head, and trunk). If the stimulus is sufficiently long-lasting, an activation of the spinal locomotor networks will occur, possibly with swimming movements toward the fictive target. The type I response could thus constitute a form of visuomotor coordination used in goal-directed locomotion.

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