Multiple Manifestations of Microstimulation in the Optic Tectum: Eye Movements, Pupil Dilations, and Sensory Priming

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Netser S, Ohayon S, Gutfreund Y. Multiple manifestations of microstimulation in the optic tectum: eye movements, pupil dilations, and sensory priming. J Neurophysiol 104: 108–118, 2010. First published April 28, 2010; doi:10.1152/jn.01142.2009. It is well established that the optic tectum (or its mammalian homologue, the superior colliculus) is involved in directing gaze toward salient stimuli. However, salient stimuli typically induce orienting responses beyond gaze shifts. The role of the optic tectum in generating responses such as pupil dilation, galvanic responses, or covert shifts is not clear. In the present work, we studied the effects of microstimulation in the optic tectum of the barn owl (Tyto alba) on pupil diameter and on eye shifts. Experiments were conducted in lightly anesthetized head-restrained barn owls. We report that low-level microstimulation in the deep layers of the optic tectum readily induced pupil dilation responses (PDRs), as well as small eye movements. Electrically evoked PDRs, similar to acoustically evoked PDRs, were long-lasting and habituated to repeated stimuli. We further show that microstimulation in the external nucleus of the inferior colliculus also induced PDRs. Finally, in experiments in which tectal microstimulations were coupled with acoustic stimuli, we show a tendency of the microstimulation to enhance pupil responses and eye shifts to previously habituated acoustic stimuli. The enhancement was dependent on the site of stimulation in the tectal spatial map; responses to sounds with spatial cues that matched the site of stimulation were more enhanced compared with sounds with spatial cues that did not match. These results suggest that the optic tectum is directly involved in autonomic orienting reflexes as well as in gaze shifts, highlighting the central role of the optic tectum in mediating the body responses to salient stimuli.

INTRODUCTION

An event that is perceived by an animal as salient typically induces a wide range of behavioral and physiological responses (Boehnke and Munoz 2008; Sokolov 1963). Although a rapid shift of gaze is the most apparent response (Dean et al. 1989), along with it a series of autonomic reflexes occur to prepare the body for possible action (Sokolov 1963). These include galvanic responses (Bradley 2009), changes in heart rate (Bradley 2009), changes in brain wave activity (Naatanen 1995), and pupillary dilation (Bala and Takahashi 2000; Oleson et al. 1972). This wide repertoire of responses, which is remarkably preserved phylogenetically, has been termed by Pavlov as the “orienting response” (Sokolov 1963). Orienting responses can include gaze shifts (overt orienting), but do not have to (covert orienting). An important question is which neurons in the brain register the saliency of the stimulus to trigger this cascade of physiological responses—a cascade that encompasses various regions of the cortex, basal ganglia, and brain stem nuclei (Boehnke and Munoz 2008; Knudsen 2007; Robinson and Petersen 1992).

It has been suggested that neurons in the deep and intermediate layers of the superior colliculus (SC) can support this function (Boehnke and Munoz 2008). The SC contains neurons that are organized to form a multisensory spatial map whose responses are correlated with the saliency of the stimulus inside their receptive field (Fecteau and Munoz 2005; Horwitz and Newsome 1999; McPeek and Keller 2002). The SC sends wide projections to cortical and basal ganglia regions via thalamic nuclei (Robinson and Petersen 1992; Takada et al. 1985) and projections to motor nuclei (Rodgers et al. 2006).

The SC is arguably one of the most phylogenetically conservative structures in the brain (Gaster and Stein 1979; Luksch 2003; Shimizu and Karten 1993). The avian homologue of the SC is the optic tectum (OT). Both the mammalian and the avian structures are laminated and demonstrate many similarities in their input and output patterns (Shimizu and Bowers 1999). Functionally, both have been shown to be involved in overt orienting to salient stimuli (du Lack and Knudsen 1990; McPeek and Keller 2004; Wagner 1993) and in multisensory processing (Stein and Meredith 1993; Zahar et al. 2009). An emerging hypothesis is that the evolutionary role of the OT/SC is to sort stimuli based on saliency and send this information to the appropriate brain regions to direct orienting movements, attention, and autonomic responses (reviewed in Boehnke and Munoz 2008; Knudsen 2007).

Many of the studies supporting the saliency hypothesis of the OT are based on electrophysiological recordings from which it is difficult to discern whether the site of recording reflects phenomena happening elsewhere in the brain or whether it contributes directly to the process at hand. In this aspect, electrical microstimulation is a powerful experimental tool that complements electrophysiological recordings by providing causal evidence (Dorris et al. 2002; Moore and Fallah 2004; Muller et al. 2005).

In this study, we used microstimulation to explore the function of the OT in barn owls. The pupillary dilation response (PDR) served as a behavioral metric. In barn owls, the diameter of the pupil increases immediately following an acoustic stimulus. However, if the same stimulus is repeatedly presented, the amplitude of the response dramatically habituates (Bala and Takahashi 2000; Bala et al. 2007). Infrared video was used to measure PDRs and eye movements in head-restrained and lightly anesthetized barn owls. We first report that low-level electrical microstimulation in the OT can...
induce PDRs independent of eye movements. The electrically evoked PDRs resembled acoustically evoked PDRs. At even lower stimulation levels, microstimulation followed by an acoustic stimulus induced a certain degree of recovery from habituation. Our findings thus suggest that the OT is directly involved in autonomic orienting reflexes as well as attention, supporting the above-cited hypothesis that the cascade of orienting responses is initiated in the OT.

METHODS

Animals

For this experiment, we used 10 barn owls (*Tyto alba*). All birds hatched in our colony at the Faculty of Medicine in the Technion. The birds were kept in large aviaries and were treated in accordance with the guidelines of the Technion Institutional Animal Care and Use Committee.

**Electrophysiological recordings**

The birds were prepared for repeated experiments with a single surgical procedure. The birds were anesthetized with 2% isoflurane in a mixture of nitrous oxide and oxygen (4:5). A craniotomy was performed and a recording chamber was cemented to the skull. At the beginning of each electrophysiological session, the bird was briefly anesthetized with isoflurane (2%) and nitrous oxide in oxygen (4:5). Once anesthetized, the animal was positioned in a stereotaxic apparatus and aligned using the pecten oculus as a retinal landmark (Wathey and Pettigrew 1989). Within the booth, isoflurane was administered with a fixed mixture of nitrous oxide and oxygen (4:5). The craniotomy cover was removed and the bird was maintained on a fixed mixture of nitrous oxide and oxygen (4:5). The electrode was driven into the OT or the lateral part of the inferior colliculus (IC). A Tucker-Davis Technologies (TDT) System 3 and an on-line spike sorter (MSD, Alpha-Omega) were used for isolation of action potentials from a small cluster of neurons (multitunit recording). At the end of each recording session, the recording chamber was treated with chloramphenicol ointment (5%) and closed. The owl was then returned to its home flying cage.

**Targeting of nuclei**

The identification of the recording sites was based on stereotaxic coordinates and on expected physiological properties. The OT was recognized by characteristic bursting activity and spatially restricted visual receptive fields (Knudsen 1982). The position within the OT was determined based on the location of the visual receptive field. Once the superficial, bursting layer of the OT was reached, the electrode was further lowered (500–1,000 μm) into the deep layers of the OT (Fig. 1, site b), which were identified based on characteristic regular firing patterns, in marked contrast to the bursting activity of the superficial layers (Knudsen 1982 and Fig. 1). All microstimulation sites were in the regular firing layers, which are known to correspond with tectal layers 11–14 (Knudsen 1982), and together were termed the deep layers of the OT. Figure 1 shows the anatomical reconstruction of an electrode penetration through the OT and two typical microstimulation sites are shown (sites b and c) together with corresponding traces of the recorded potentials. Recording sites were reconstructed by passing a positive current of 5 μA for 30 s. Seven days after the electrolytic lesions the owl was deeply anesthetized with isoflurane and nitrous oxide/oxygen and given a dose of heparin (0.3 ml) and nembutal (0.5 ml) injected directly into the left ventricle. Owls were then perfused through the heart with 250 ml of 0.4 M phosphate buffer (PO4, pH 7.4) followed by 250 ml of 4% formaldehyde solution. The brain was fixed in paraffin blocks and transversely sliced in 10 μm sections. Sections were then mounted onto Superfrost Plus glass slides and Nissl stained.

The lateral part of the IC was targeted by positioning the electrode 2 mm caudal and 2.5 mm medial from the tectal representation of 0° azimuth and +10° elevation (relative to the visual axes). The electrode was then moved laterally and rostrally in steps of about 300 μm to sample additional sites from the IC. In previous studies, anatomical reconstructions of recording sites confirmed the correspondence of these stereotaxic coordinates with the lateral parts of the IC (external nucleus and lateral shell) (Brainard and Knudsen 1993; Gold and Knudsen 2000; Gutfreund et al. 2002). Within the IC, only sites with physiological characteristics of the external nucleus of the inferior colliculus (ICX; Brainard and Knudsen 1993; Gutfreund and Knudsen 2006) were used for microstimulation.

**Electrical microstimulation**

A stimulus isolator (A365R; WPI) was used to deliver electrical stimulation to the OT. One pole of the stimulus isolator was connected to the electrode (the same electrode used for recording) and the other to the stainless steel recording chamber. In all experiments, trains of 200 μS biphasic stimuli at a rate of 200 Hz for 80 ms (15 stimuli per
pulse train) were used. The current level varied between 5 to 150 μA. Microstimulations were repeated at interstimulus intervals (ISIs) >10 s. In experiments in which the microstimulation was coupled to an auditory stimulus, the microstimulation ended at the onset of the auditory stimulus. In all cases the electrode for microstimulation was positioned in the right OT.

Auditory stimulation

Computer-generated signals were transduced by a pair of matched miniature earphones (Knowles ED-1914). The earphones were placed in the center of the ear canal about 8 mm from the tympanic membrane. The amplitude and phase spectra of the earphones were equalized within ±2 dB and ±2 μs between 2 to 12 kHz by computer adjustment of the stimulus waveform. Acoustic stimuli consisted of 600 or 200 ms bursts of either broadband (3–10 kHz) or single tones. Sound levels were controlled by two independent attenuators (TDT PA5). Unit responses to an auditory stimulus were quantified as the number of spikes in a given time window after stimulus onset minus the number of spikes during the same time window immediately before stimulus onset (baseline activity). Interaural time difference (ITD) tuning curves were generated by presenting a series of sounds in which each sound had a different ITD. All other acoustic parameters were maintained constant. The ITD value was varied randomly in stimulus sets that were repeated 10–20 times. The best ITD was defined as the midpoint of the range over which responses were >50% of the maximal response. Interaural level difference (ILD) curves were generated using the same procedure, but varying the ILD of the sound.

To measure habituation of acoustically induced PDRs, a sequence of 10 similar auditory stimuli with an ISI of 12 s was presented from a loudspeaker positioned 1.5 m from the owl. This sequence was repeated up to five times during a single experimental day. In each repetition, the position of the loudspeaker and the frequency of the sound were changed (in a range of left 30° to right 30° and 3–10 kHz) to minimize cross-session habituation. We did not observe any qualitative differences in the PDRs to the different auditory stimuli.

Measuring pupil responses

Pupil responses were measured while the owls were positioned in the electrophysiological setup described earlier. The right eye was kept open by attaching miniature clips to the small feathers on the eyelids. The clips were gently pulled by strings to open the eyelid. This procedure did not prevent blinking of the nictitating membrane (allowing for spontaneous moisturizing of the eye). An analog video camera (Wat-902DM2s, Watrec, 25 Hz) equipped with a zoom lens (Pentax C-mount TV lens, 75 mm, F-1:2.8 fitted with ×2 extender) was positioned about 1 m from the owl’s head. An infrared (IR) light-emitting diode (LED) was attached to the camera lens as close as possible to the camera axes. The camera and LED positions were adjusted manually in each session to optimally capture the IR light reflected from the retina and to obtain a high contrast image of the pupil (Fig. 2A and B). The camera angle relative to the head was adjusted so that the lateral edge of the pecten oculus was visible (Fig. 2B, arrow). Once the camera was positioned in place, the door of the booth was closed and video sequences of the pupil were collected starting 1 s before the onset of the stimulus and ending 3 or 7 s after. Video sequences were analyzed off-line using two approaches. The direct approach fitted a circle to the pupil in each video frame (a detailed description of the algorithm can be found in the on-line Supplemental material).1 The diameter of the circle and its horizontal position were registered (Fig. 2C, red and black curves). The edge of the pecten (depicted as a blue curve in Fig. 2A) was also segmented and the mean horizontal value of the curve points was used to define the pecten edge position (Fig. 2C, blue curve). In the example shown in Fig. 2, electrical microstimulation (gray bars in Fig. 2, C and D) induced eye movement, which can be seen by the horizontal shift of the center of the pupil immediately following the stimulation (Fig. 2C, black curve). The same eye movement was better reflected by the horizontal movement of the edge of the pecten (Fig. 2C, blue curve). The larger signal given by the pecten compared with the center of the pupil is expected because the pecten is viewed through the magnifying lens of the eye. Thus by looking at pecten movements rather than the

1 The online version of this article contains supplemental data.

FIG. 2. Analysis of video sequences. A: an example of a single video frame of the pupil. The red circle was fitted to the edge of the pupil using the algorithm described in the Supplemental material. The black cross designates the center of the circle and the blue curve is the automatically detected edge of the pecten. B: another example of a single video frame of the pupil. The red square shows the window of analysis overlapping the pupil edge. The blue square shows the window overlapping the pecten edge. The inset is a binary image of the same video frame. The white arrow points to the pecten edge. C: results from a single trial analyzed using the direct approach. Curves were smoothed for display (5-point running average). The red curve shows the radius of the pupil as a function of time. The blue curve shows the horizontal position of the pecten edge as a function of time (negative is a leftward shift). The black curve shows the horizontal position of the pupil center. The baselines of the curves were spaced along the y-axis for viewing purposes. The vertical gray bar designates the time of electrical microstimulation (80 ms). D: results from a single trial, analyzed using the nondirect approach. Curves were smoothed for display (5-point running average). The red curve shows the number of white pixels as a function of time inside the red window in B (corresponding to pupil movements). The blue line designates the number of white pixels inside the blue window (corresponding to pecten movements). The vertical gray bar designates the time of electrical microstimulation (80 ms). Data in C and D were extracted from the same trial.
center of the pupil, we can increase the sensitivity of detecting small eye shifts.

The second, indirect approach involved converting the grayscale image to a binary image using a manually set threshold and counting the number of pixels above the threshold in a manually defined rectangle that overlaps with either the pupil edge or the pecten edge (Fig. 2B, red and blue rectangles, respectively). A signal corresponding to pupil diameter was obtained by the number of pixels above the threshold in the first rectangle (Fig. 2D, red curve). In a similar manner, a signal corresponding to eye movement was obtained from the rectangle overlapping the pecten area (Fig. 2D, blue curve). Measurements obtained from the two approaches were qualitatively similar (compare Fig. 2, C and D). The advantage of the indirect approach was that it allowed us to measure pupil dilation in sessions in which part of the pupil was covered by an eyelid, many of which were cases in which the circle-fitting algorithm failed. Therefore most of the analysis in this study was performed using data from the indirect approach and only Figs. 2 and 3, A–D contain data analyzed using the direct approach.

In all experiments, the same settings as in the example shown were maintained (i.e., the right eye and the right edge of the pecten were filmed). Thus a positive shift in the pupil center (rightward shift on the image) corresponds with a leftward movement of the eye (nasal movement). Likewise, a negative shift of the pecten edge corresponds with a leftward movement of the eye. (The pecten is attached to the retina; thus a leftward rotation of the eye shifts the retinal landmark in the opposite direction.) Note that in this study, the system was not calibrated and therefore we do not know the amplitude in angles of the eye shifts. Since eye movements in barn owls are limited to roughly ±3° (du Lac and Knudsen 1990), the signals measured in this study reflect small eye deviations not >3°.

Data analysis

All trials containing eye blinks, nictitating membrane movements, or unstable pupil diameter during the prestimulus time window (1 s) were discarded from the analysis (~35% of all trials). Eye blinks and nictitating membrane movements were identified manually. Trials with unstable pupil diameter were identified automatically by measuring the mean and SD of the pupil diameter signal in two time intervals: [−1,000, −500] and [−500, 0] ms relative to stimulus onset. A trial was excluded if the difference between the means of the two windows was larger than twice the average SDs. Empirically, we found this criterion to be a good indicator of trials containing unstable pupil diameter in the prestimulus time.

To quantify the PDR, we first reduced the response curve (pupil diameter as a function of time) to baseline (i.e., subtracting the average baseline activity in the interval [−1,000, 0] ms). The PDR magnitude was then defined as the sum of the pupil diameter values during the poststimulus time window (i.e., area under the response curve). In experiments in which the indirect approach was used, the PDR magnitude was computed in a similar manner by replacing the pupil diameter values with the pixel count inside the rectangle overlapping the pupil edge.

To quantify the separation of the responses to microstimulation from the baseline activity or from the responses to auditory stimuli, a receiver operating characteristic (ROC) analysis was used. ROC curves were obtained by varying in small steps the threshold from the lowest data point to the highest data point. At each step, the proportion of responses to microstimulation that exceeded the threshold (“hits rate”) versus the proportion of responses to no-stimulus (or to auditory stimulus) that exceeded the threshold (“false alarms rate”) was plotted (example in Fig. 3, C and D). The area under the ROC curve (ROC value) can vary from 0.5, indicating a chance level, to +1 or −1, indicating perfect separation. To test the null hypothesis that the ROC value is equal to 0.5 we applied a randomized approach (Hooton 1991); from the larger population of control responses (PDRs measured in no-stimulus condition) a subpopulation equal in size to the number of responses to microstimulation was randomly selected and its ROC value relative to the remaining control responses was obtained. This procedure was repeated 5,000 times. If the ROC value of the responses to microstimulation exceeded 0.5 ± 1.64 × SD of the resample distribution, the null hypothesis was rejected at a confidence level of 5%. When it was necessary to compare two populations with an equal number of measurements, we used a bootstrap technique (sampling with repetitions). A nonparametric method that does not require a priori assumptions (ROC analysis and the randomization statistics) was chosen because the distribution of the response magnitudes did not seem to follow a normalized distribution: in a single experiment not all stimulations induced responses (see, e.g., Fig. 3C).

![FIG. 3. Pupil dilations and eye shifts evoked by microstimulation.](http://www.jn.org)
RESULTS

Effects of microstimulation in the OT

The effects of microstimulation in the deep layers of the OT on eye movements and pupil diameter were studied. In the example shown in Fig. 3A, microstimulation of 150 µA was applied to the right OT while the right eye was filmed with an IR video camera. In this example, the microstimulation induced an eye shift (Fig. 3A, black curve). In addition, a clear increase in the diameter of the pupil was observed (Fig. 3A, red curve). An initial transient dilation was followed by a long-lasting dilation. Maximal dilation was only 5% from the original pupil diameter, but in terms of pixels on the camera plane, it was much larger than the eye movement (compare black curve with red curve in Fig. 3A). Figure 3B shows results from microstimulation at the same tectal site but at a lower current level of 50 µA. Here, no eye movement was observed, even at the more sensitive measurement of the pecten position (Fig. 3B, black and blue curves). The pupil, on the other hand, responded to the weaker microstimulation with a long-lasting dilation (Fig. 3B, red curve). Video examples of pupil responses with and without eye movements can be viewed in the Supplemental material.

To quantify this response we repeated the 50 µA microstimulation 10 times with an ISI of 60 s. For each microstimulation we measured the PDR magnitude (see METHODS). In addition, PDRs were measured every 12 s between microstimulations (control responses)—four control measurements between two test measurements. Figure 3C shows the pupil responses to microstimulation (black diamonds) together with the pupil responses in control trials (gray diamonds). Note that some data points are missing because of blinks and/or unstable base levels (see METHODS for selection criteria). Pupil responses to the microstimulation were generally above the 95% confidence interval of the control responses (dashed line). To evaluate the separation between the responses to microstimulation and the control trials, ROC analysis was used (see METHODS). Figure 3D shows the ROC curve obtained from the data in Fig. 3C. In this case the ROC value (0.87) was significantly above chance level (randomization test; \( P < 0.01 \)).

The experimental session described in Fig. 3 was repeated 80 times in 32 different tectal sites using a range of different stimulation levels. The ROC analysis and the randomization statistics were used to separate all the experiments into four subgroups: 1) experiments in which the ROC curves for both pecten and pupil measurements were significantly above chance (positive eye movements and positive PDR; gray bars in Fig. 3E); 2) experiments in which only the ROC curve of the pupil edge was significantly above chance (positive PDR; red bars in Fig. 3E); 3) experiments in which only the ROC curve of the pecten edge was significantly above chance (positive eye movement; blue bars in Fig. 3E); and 4) experiments in which none of the measurements was significant (total number of experiments minus the sum of all bars). The proportion of sites with positive eye movements or PDRs increased from 36% at 20–30 µA to 100% at 150 µA. Among the experiments that were negative for eye movements (observed at current levels <100 µA), 37% had a positive PDR. For experiments that were positive to eye movements (observed at all current levels) 37% showed no significant PDR. Thus it was possible to evoke PDRs without eye movements and vice versa.

The population average response curves of all four groups are shown in Fig. 4. Clear average PDRs were observed at the four current levels (left column). Evoked pecten movements, on the other hand (right column), ranged from an almost unnoticeable negative shift at current levels of 20–30 µA (Fig. 4B) to a robust signal at current levels of 100 µA (Fig. 4H). Note that the scale of the y-axis in Fig. 4, A, B, C, and D is enlarged compared with that in Fig. 4, E, F, G, and H (black scale bar on the y-axis). Following the initial negative shift of the pecten edge, a positive average shift was seen in most cases (Fig. 4, D, F, and H). This, we believe, does not reflect rightward eye shifts but rather an influence of the pupil dilation on the pecten measurement. As the pupil dilates, more light enters the eye and the pupil image becomes brighter. Thus a retreat of the dark edge of the pecten is expected purely due to the increase in brightness. This optical artifact was small and revealed only in average traces.

![Figure 4](http://jn.physiology.org/DownloadedFrom/10.1152/jn.00553.2009)

**FIG. 4.** Average response profiles of all experiments. The *left column* (A, C, E, and G) shows the average pupil responses to microstimulation at 4 different current levels. The *right column* (B, D, F, and H) shows the average pecten responses to microstimulation at the different current levels. The vertical gray bars designate the duration of microstimulation and the black horizontal bars indicate the y-axis scale. (Note that the scale in A, B, C, and D is fourfold larger than that in E, F, G, and H.)
Comparison of electrically evoked PDRs with acoustically evoked PDRs

We elicited and measured acoustically evoked PDRs to compare them with responses evoked by tectal microstimulation. It should be noted that acoustically evoked PDRs in the barn owl were previously explored and characterized in detail (Bala and Takahashi 2000; Bala et al. 2003, 2007; Spitzer et al. 2003). However, in the current work barn owls were under the influence of nitrous oxide during the microstimulation. Therefore, for the comparison, it was necessary to measure acoustic PDRs in the barn owl were previously explored and characterized in detail (Bala and Takahashi 2000; Bala et al. 2003, 2007; Spitzer et al. 2003). However, in the current work barn owls were under the influence of nitrous oxide during the microstimulation. Therefore, for the comparison, it was necessary to measure acoustic PDRs in the current work.

In a second set of experiments the auditory stimuli were substituted by microstimulations in the deep layers of the OT (Fig. 5, C and D). This experiment was repeated with eight different stimulation sites (five repetitions in each site) in four owls. The current level in all cases was 30 μA. The electrically evoked PDRs also habituated when the stimulus was presented repeatedly (Fig. 5C); however, the habituated responses maintained an average level above zero, even after 10 repetitions of the stimulus (Fig. 5D).

To compare the time course of the auditory evoked response with that of the electrically evoked response, the responses were measured over a longer duration (7 s compared with the 3 s window used earlier). In these experiments, auditory and electric stimulation were presented with an ISI of 60 s to minimize habituation (20–30 repetitions of each type of stimulus). The experiment was repeated at three recording sites in one owl. The average PDR to all auditory stimuli is shown in Fig. 5E (solid line) together with the average PDR to the microstimulation (dashed line). Both types of stimulation induced average responses that lasted more than the measured 7 s.

**PDRs evoked by microstimulation in the external nucleus of the inferior colliculus**

Following the finding that microstimulation in the OT could induce PDRs, we extended our experiments into the ICX, the source of auditory input to the OT (Gold and Knudsen 2001). PDRs were measured while applying microstimulation in ICX sites from two different owls. In each site we applied sequences of 10 stimuli with an ISI of 60 s and measured the PDRs. In addition, we measured control trials between stimuli and the measured 7 s. Two levels of stimulation were used: 20–30 μA in 20 different recording sites and 50–80 μA in 28 different recording sites. To pool data from different experiments we first normalized the PDR magnitudes to Z-scores by subtracting the mean from every data point and dividing by the SD of the control trials. Z-scores of all tests with similar conditions were pooled into a single data set to obtain the ROC value. In addition, for display purposes, Z-scores were presented as averages per stimulus position in the sequence (Fig. 6, A and C). In the experiments in which the stimulus level was 20–30 μA, the ROC value of the responses to microstimulation (Fig. 6A, black diamonds) relative to control trials (gray diamonds) was not significantly >0.5 (randomization method; P > 0.05). The average response pattern to the 20–30 μA microstimulation (black curve in Fig. 6B) is shown together with the average of the control trials (gray curve in Fig. 6B). It can be seen that the average PDR to microstimulation at the low current level was only slightly above the control. Stronger current levels in the ICX (50–60 μA), however, induced distinct PDRs. The average Z-scores are shown in Fig. 6C. Here the ROC of the Z-scores was significantly >0.5 (randomization method; P < 0.05). The population average PDR evoked by the higher level of microstimulation in the ICX is shown in Fig. 6D (black line). It can be seen that the ICX evoked PDRs were also long-lasting, exceeding the measured 3 s. In contrast to tectal sites, microstimulation in the ICX sites did not induce detectable eye movements. Note, however, that the maximal stimulation level used in the ICX was 80 μA; it is possible that higher stimulation levels were effective for producing eye movements.

**FIG. 5.** Comparison between PDRs to tectal microstimulation and PDRs to acoustic stimuli. A: average PDR profiles to acoustic stimulation. The solid line is the average response to the first stimulus in the sequence. The dashed line is the average response to the first stimulus in the sequence. The horizontal bar indicates the duration of the stimulus (600 ms). B: a graph showing the average acoustic PDR magnitude as a function of the position of the stimulus in the sequence. The error bars indicate SEs. The low diamond symbols designate data points that are significantly smaller than the first data point (t-test; P < 0.05). C: average PDR profiles to microstimulation. The solid line is the average response to the first microstimulation in the sequence. The dashed line is the average response to the first microstimulation in the sequence. The error bars indicate SEs. The low diamond symbols designate data points that are significantly smaller than the first data point (t-test; P < 0.05).
average pecten responses to the auditory stimulation as well as to the electrical stimulation demonstrated a weak initial negative shift (Fig. 7C, gray and blue curves, respectively). The coupled stimulus on the other hand gave rise to a larger negative shift. An ROC analysis performed over the Z-scores of the response magnitudes revealed that this enhancement was significant (bootstrap; \( P < 0.05 \), suggesting that the coupled stimulus induced eye shifts toward the stimulus direction more readily than its components alone.

An important question is whether the enhancement effect is specific to sounds with ITDs encoded at the focal location of the microstimulation. To answer this question, we presented, in another set of experiments, two auditory stimuli: one with ITD and ILD values matching the best ITD and ILD of the electrode site (matched stimulus) and the other with ITD and ILD values, yielding minimal responses in the tuning curves of the site (nonmatched stimulus). The two sounds were presented, first alternating every 5 s for 200 repetitions to habituate the responses to both stimuli (responses to the first 200 stimuli were omitted from the analysis). Then, a low-level microstimulation was coupled with every fifth stimulus (starting at stimulus 205), once coupled to its matched and once to its nonmatched auditory stimulus (see time course of experiment in Fig. 8A). Figure 8B shows the population average response to the four conditions (matched coupled stimulation, nonmatched coupled stimulation, matched auditory stimulation, and nonmatched auditory stimulation). The average responses to both auditory stimuli were strongly habituated (Fig. 8B, gray curves). Moreover, they showed a tendency to decline. However, we believe this is an outcome of our criterion to remove trials in which the baseline (first 1 s) was not stable (see METHODS) together with spontaneous pupil constrictions in the data set. The average responses including all trials did not show this tendency. In contrast to the auditory stimuli, the

**Coupling of low-level microstimulation with acoustic stimulation**

In the following experiments, we measured the interactions between low-level microstimulation in the OT (8–20 \( \mu A \)) and auditory stimulation. In this set of experiments, a sequence of 100 auditory stimuli was presented with an ISI of 12 s to induce habituation of the acoustic PDR. Importantly, the ITD and ILD of the auditory stimuli matched the values that were best represented at the stimulation site (see METHODS). Responses to the first 100 stimuli were omitted from the analysis. The initial habituating sequence was followed by 10 repetitions of a sequence composed of four auditory stimuli, a single electrical stimulus, four additional auditory stimuli, and a single coupled electrical and auditory stimulus (see diagram in Fig. 7A). An ISI of 12 s was maintained throughout the sequence. The experiment was repeated 25 times in one barn owl. The population averages of the pupil responses are plotted in Fig. 7B. The auditory stimulation induced, due to habituation, a small average PDR (Fig. 7B, gray curve). Despite the low levels of microstimulation used in this experiment, a clear PDR to the microstimulation alone was observed in the average profile (Fig. 7B, blue curve). Coupling of the two stimuli induced an average PDR curve (Fig. 7B, red curve) that was above the PDRs induced by each stimulus presented alone (microstimulation or auditory). Note that the difference was most apparent at longer post stimulus times. A bootstrap method, applied to assess the differences between the response magnitudes to the coupled stimulus and the microstimulation alone, did not show a significant difference (\( P = 0.16 \)). Interestingly, pecten movements, measured in the same experiments, displayed a similar and more significant tendency.

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**Fig. 6.** PDRs evoked by microstimulation at external nucleus of the inferior colliculus (ICX) sites. **A:** the average Z-scores of the population of all stimulation sites in the ICX are shown. Black diamonds designate average responses to microstimulation at current levels of 20–30 \( \mu A \). The gray diamonds represent the average Z-scores obtained in control trials. **B:** the population average response profile to microstimulation at current levels of 20–30 \( \mu A \) (black line) is compared with the population average of the control trials (gray line). **C** and **D:** responses of the same recording sites as in **A** and **B** but using higher current levels (50–80 \( \mu A \)). Format as in **A** and **B**. The scale of the y-axis in **B** and **D** is the same.

**Fig. 7.** Coupling of acoustic and tectal electrical stimulation. **A:** the time course of the stimulation protocol used in this experiment. Gray vertical bars designate auditory stimuli and blue vertical bars designate electrical stimuli. **B:** population PDR profiles showing the average response to the habituated auditory stimulus (gray curve), to the microstimulation alone (blue curve), and to the coupled microstimulation and auditory stimulus (red curve). The vertical gray bar indicates the time and duration of the electrical stimulation and the horizontal black bar the time and duration of the auditory stimulus (200 ms). **C:** the population averages of pecten response profiles measured during the same experiments used in **B**. Format and colors as in **B**.
The matched coupled stimuli and the nonmatched coupled stimuli. Only sessions in which a significant difference was observed ($P < 0.05$) in any direction were used for the analysis (8 experiments of 27). If the interaction is not specific to sounds encoded at the location of the stimulus, the pooling of responses from all 8 tests should give no difference between matched and nonmatched stimuli. However, this was not the case. The average PDR to the matched stimuli, in this sub-population of sites, was above the average PDR to the non-matched stimuli (Fig. 8, C and D). This difference was statistically significant (bootstrap, $P < 0.05$). Thus, we conclude that the response to the coupled stimulus was influenced by the spatial location encoded at the stimulation site.

**Discussion**

**OT and the orienting response**

Microstimulation in the deep layers of the OT was capable of evoking two distinct responses: eye movements and PDRs. The relatively low current levels of microstimulation used here and the fact that both effects could be induced from the same stimulation sites suggest that the same local circuitry triggers both outcomes. Behavioral and physiological assessments of the effective current spread during microstimulation with 20 $\mu$A (current levels that were capable of evoking PDRs in this study; Figs. 4A and 7) suggest that neurons $\approx 200$ $\mu$m from the electrode tip are directly activated (Tehovnik et al. 2006). This estimation makes it unlikely that the microstimulation used here, with electrodes well within the OT (Fig. 1), directly activated neural elements outside of the OT. Still, since the OT receives multiple inputs from the forebrain (Bravo and Pettigrew 1981; Knudsen et al. 1995), it is possible that the evoked PDRs may be a consequence of antidromic activation through presynaptic fibers from an unknown brain area. However, microstimulation in ICX sites induced a similar PDR. The ICX provides, exclusively, ascending auditory information to the OT (Arthur 2005; Knudsen and Knudsen 1983). Thus it is most likely that the PDRs evoked by ICX microstimulation were mediated through ICX-tectal projections, giving rise to activation of tectal neurons and not to antidromic activity. It is interesting to note that the same microstimulations in the ICX did not induce detectable eye movements, a result that suggests a separation in the OT between the PDR pathway and the eye movement pathway. The first may require less activation to be effective or an activation of a separate population of neurons that are better coupled with the ICX.

Microstimulation in the OT of a wide variety of species is known to evoke head and eye movements toward the location that is represented at the site of stimulation. To name a few: barn owls (Masino and Knudsen 1990), cats (McHaffie and Stein 1982), primates (Sparks 1986), gold fish (Herrero et al. 1982), and iguana (Stein and Gaither 1983). These observations contributed to establish the highly conserved role of the OT in integrating spatial information and controlling gaze direction (Sparks and Hartwich-Young 1989). However, why would microstimulation in the OT result in PDRs? This is not an obvious outcome of gaze control. Perhaps the answer is that the OT is involved in more than just gaze control. Both evoked responses (PDRs and eye shifts) constitute responses to salient stimuli, i.e., they are part of the general orienting response.
An additional effect of low-level microstimulation at the same tectal sites was priming—i.e., the pupil response to an already habituated stimulus tended to increase when the habituated stimulus came after an electrical microstimulation. This result was statistically weak, possibly reflecting the substantial PDRs to low-level microstimulation alone. However, the corresponding result that a similar priming effect was observed in the eye movements (Fig. 7C) reinforces the priming effect. The increased response cannot be attributed to general arousal effects or to simple summation of independent responses to two stimuli because it was dependent on the spatial location (in ITD/ILD space) of the region encoded at the stimulation site (Fig. 8). The cellular mechanisms of this effect are unknown. It is possible that the microstimulation directly affects the membrane potential of the principal cells for a short period of time, allowing later arriving auditory inputs to more readily cross the threshold and thereby induce an overall enhanced behavioral response. In several aspects this finding resembles the results reported by Muller et al. (2005): improvement of a behavioral response to a sensory stimulus at a location corresponding with the location of a brief microstimulation in the SC. The interpretation of Muller et al. (2005) to their priming effect was that microstimulation at a specific site mimics the reallocation of attention to the corresponding location. Priming or cueing effects are part of the typical response to salient stimuli (Itti and Koch 2000; Posner 1980; Sokolov 1963). Importantly, behavioral cueing effects have been shown in barn owls (Johnen et al. 2001). Thus the three effects of microstimulation reported here—eye movements, PDRs, and space-specific priming—are all consistent with the orienting response.

An alternative interpretation of our data is that tectal microstimulation created a perception of a sensory event similar to the phosphene phenomenon (Nashold Jr 1970). In principle, a phosphene can be perceived as a salient event, thereby indirectly inducing the orienting responses. To rule out such a possibility is impossible because we can never know what the animal actually perceives. However, it should be emphasized that the barn owls in this study were lightly anesthetized, a situation in which we expect phosphene perception to be suppressed.

Comparison with other species

Interestingly, an early study reported that electrical stimulation in the OT of birds can induce pupil dilation (Showers and Lyons 1968). The pupil response was not quantified and was evoked by nonfocal high current stimulations. Nevertheless, this early observation suggests that the pupil dilation effect we report here is not unique to barn owls; it exists in other avian species as well. However, what about mammals? One hypothesis is that in birds, which do not possess a layered neocortex (Northcutt and Kaas 1995), the OT has a broader function in sensory processing compared with mammals. This hypothesis would suggest different effects of tectal microstimulation between avian and mammalian species. However, we find this hypothesis less appealing. The role of the OT in selecting behaviorally relevant stimuli was previously shown to take place in amphibians (Ingle 1975). It seems unlikely that the brain of mammals evolved completely different structures to take over this basic function. This view is supported by a large body of literature pointing to remarkable functional and anatomical similarity between avian and mammalian brains, including pathways that connect the OT and the forebrain (reviewed in Jarvis et al. 2005). We therefore predict that, if searched for, evoked PDRs by tectal microstimulation may also be observed in mammalian species. Indeed, a previous study anecdotally reported pupil dilation in response to electrical stimulation in the macaque SC (Jampel 1960).

If the hypothesis that signals in the OT initiate the cascade of orienting responses is true, and that this function is conserved in the mammalian SC as well, we expect microstimulation in the SC to induce other orienting responses in addition to PDR, such as vascular reactions and changes in electroencephalographs (EEGs), for instance. Evidence that this prediction is true can be found in the literature. Microstimulation in the SC induces responses in the electromyographic activity of neck muscles independent of eye or head movements (Cornell et al. 2007). In addition, microstimulation in the SC induces ocular accommodation (Sawa and Ohtsuka 1994), freezing responses (Dean et al. 1989), increased heart rate (Keay et al. 1988), arousal in cortical EEG (Redgrave and Dean 1985), and suppression of eye blink reflex (Basso et al. 1996; Gnadt et al. 1997). Taken together, it is clear that microstimulation in the SC can induce a wide range of physiological responses that do not shift the gaze of the animal. Importantly, the above-cited examples constitute responses to salient sensory events (Bradley 2009; Powers et al. 1997; Sokolov 1963; Yeomans and Frankland 1995).

Possible pathways

What is the anatomical pathway that mediates the electrically evoked PDR? The same pathway is likely to provoke sensory evoked PDRs. One possible pathway was suggested by Bala and Takahashi (2000). The OT of the barn owl sends projections to the medial mesencephalic reticular formation (MRF; Masino and Knudsen 1992). The MRF sends axons to the accessory oculomotor complex (Gamlin and Reiner 1991), which in turn projects to the ciliary ganglion (Reiner et al. 1991), a ganglion that controls, in part, the pupil dilator muscle (Pilar et al. 1980). Another source of projection to the accessory oculomotor complex in birds is the suprachiasmatic nucleus of the hypothalamus (Gamlin et al. 1982). Thus in principle, information from the OT can also reach the ciliary ganglion though tecto-thalamic-hypothalamic tracts (Cantwell and Cassone 2006; Gamlin and Cohen 1986). Thus anatomical links exist for direct contribution of tectal neurons to pupil dilation. However, it is not known whether the tectal recipient neurons in the MRF or in the thalamus are functionally linked with the accessory oculomotor complex and thus these remain hypothetical pathways.

The long-lasting pupil dilation response and the relatively long memory of habituation (>12 s) observed in both the electrically and the sensory evoked PDRs (Fig. 5 and Bala and Takahashi 2000) suggest that at least part of the PDR involves an additional indirect pathway through the forebrain. The
forebrain arcopallium (considered homologue to the frontal eye field in primates) sends efferent fibers to the MRF (Knudsen et al. 1995). Thus a pathway carrying information from the OT to forebrain areas may play a role in mediating the PDR, exclusively or in parallel with the descending pathways. In this aspect the tectofugal pathway is one interesting possibility (Karten 1969). The pathway ascends from the OT to the nucleus rotundus (Benowitz and Karten 1976) of the dorsal thalamus, which in turn projects to a nidopallial area called the entopallium (Karten and Hodos 1970). Recent studies suggest that the avian tectofugal pathway carries information about the saliency of the stimulus (Marin et al. 2007; Reches and Gutfreund 2009; Reches et al. 2010). Moreover, it is considered to be homologous to the mammalian colliculo-pulvinar-cortical pathway (Karten 1969; Karten and Shimizu 1989; but see Guirado et al. 2005 for an alternative view), which has been suggested to play an important role in the coordination of spatial attention between the SC and the cortex (Olshausen et al. 1993; Robinson and Petersen 1992; Shipp 2004). The fact that microstimulations as low as 10–20 μA were capable of inducing PDRs suggests that the effect is mediated by the activation of large neurons in the OT (Tehovnik et al. 2006). The tecto-rotundal tract is composed of axons from tectal ganglion cells of layer 13 (Marin et al. 2003), the largest neurons in the deep layers of the OT (Luksch et al. 1998). Thus this pathway may contribute not only to the PDRs recorded here but also to other typical orienting responses.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

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