

A. D. S. Bala · T. T. Takahashi

Pupillary dilation response as an indicator of auditory discrimination in the barn owl

Accepted: 28 February 2000

Abstract The pupil of an awake, untrained, head-restrained barn owl was found to dilate in response to sounds with a latency of about 25 ms. The magnitude of the dilation scaled with signal-to-noise ratio. The dilation response habituated when a sound was repeated, but recovered when stimulus frequency or location was changed. The magnitude of the recovered response was related to the degree to which habituating and novel stimuli differed and was therefore exploited to measure frequency and spatial discrimination. Frequency discrimination was examined by habituating the response to a reference tone at 3 kHz or 6 kHz and determining the minimum change in frequency required to induce recovery. We observed frequency discrimination of 125 Hz at 3 kHz and 250 Hz at 6 kHz – values comparable to those reported by others using an operant task. Spatial discrimination was assessed by habituating the response to a stimulus from one location and determining the minimum horizontal speaker separation required for recovery. This yielded the first measure of the minimum audible angle in the barn owl: 3° for broadband noise and 4.5° for narrowband noise. The acoustically evoked pupillary dilation is thus a promising indicator of auditory discrimination requiring neither training nor aversive stimuli.

Key words Habituation · Frequency discrimination · Minimum audible angle · Sound localization · Psychoacoustics

Abbreviations *az* azimuth · *el* elevation · *MAA* minimum audible angle · *MRF* mesencephalic reticular formation · *PDR* pupillary dilation response · *SNR* signal-to-noise ratio · *SPL* sound pressure level ·

SPL_A A-weighted sound pressure level · Δf frequency difference

Introduction

Animals orient in the direction of novel stimuli. Primates, for example, make saccadic eye movements toward visual and auditory stimuli (Jay and Sparks 1987). Similarly, owls rapidly turn their heads to face auditory sources (Knudsen et al. 1979). The term “orienting response”, originally coined by I.P. Pavlov (as quoted in Sokolov 1963), however, is not limited to such overt movements of the eyes, head, or body. Orienting responses also include physiological responses such as changes in skin conductance, respiration rate, or heart rate, and pupillary dilation. Orienting responses, including the pupillary dilation response (PDR), have been applied to the analysis of a wide variety of auditory discriminations in a variety of species (reviewed comprehensively by Graham 1973). For example, frequency discrimination has been studied in humans and monkeys using the galvanic skin response (Bagshaw et al. 1965; Bagshaw and Benzie 1968; Hare 1968; Korn and Moyer 1968) or the heart rate response (Weisbard and Graham 1971). In particular, the PDR has been utilized to study auditory discrimination in cats (Oleson et al. 1972) as well as in humans (Maher and Furedy 1979; van Olst et al. 1979).

To date, all assays of auditory behavior in the barn owl (*Tyto alba*) have relied on operant conditioning tasks. For example, spatial hearing has been assessed by the accuracy with which they strike concealed speakers (Payne 1971; Konishi 1973) or by the accuracy with which they turn their heads toward a sound source (Knudsen and Konishi 1979; Knudsen et al. 1979). Frequency discrimination in the owl has been studied using a Go/NoGo task in which owls were taught to fly to a feeder when a tone of a frequency different from that of a memorized reference tone was presented (Quine and Konishi 1974).

A. D. S. Bala (✉) · T. T. Takahashi
Institute of Neuroscience, University of Oregon,
Eugene, OR 97403, USA
e-mail: avinash@uoneuro.uoregon.edu
Tel.: +1-541-346-4544; Fax: +1-541-346-4548

Although the behavioral studies employing these methods have yielded valuable insights into the owl's perceptual capabilities, they require extensive training. Moreover, these tasks require movements such as flight or rapid head turns that may make them difficult to integrate with neural recordings. In this paper, we report a new orienting response in the barn owl, pupillary dilation, evoked by changes in the bird's auditory environment. This orienting response is evoked without training in awake, head-restrained birds. Repeated presentation of a stimulus habituates the pupillary dilation, but changes in the signal-to-noise ratio (SNR), frequency, or location of the stimulus cause it to recover. We first describe the pupillary response of the owl and the conditions in which it is evoked, habituated, and recovered. We then assess its specificity for frequency and space, deriving, respectively, a psychometric function for frequency discrimination and the MAA. These measures suggest that the PDR is equivalent in sensitivity to the operant procedures described above.

Materials and methods

Subjects

The subjects were five barn owls 2–3 years old from a captive breeding colony on the University of Oregon campus. A surgically implanted headplate allowed us to fix the bird's head in a headholder at a consistent location relative to the sound source(s). Surgical procedures are described elsewhere (Takahashi and Keller 1994). After headplating, an owl was allowed to recover in an isolated recovery chamber for a minimum of 2 weeks or until the incision had completely healed, and new feathers concealed the headplate. The owl was then transferred to a shared flight space with two or three other birds.

Behavioral procedures

A newly headplated bird was acclimatized to being immobilized in an anechoic chamber over three or four sessions. Each day, the bird was placed in the headholder for progressively longer periods of time up to 1 h. During these acclimatization sessions, feathers on the upper and lower eyelids of the right eye were clipped close to the skin and the bird was familiarized to its eyes being held open by a thread taped to each eyelid.

Birds properly restrained and acclimatized to their surroundings lay still for periods ranging from 1.5–2 h. A behavioral session was temporarily halted if the bird struggled, and terminated if the bird continued to struggle. Behavioral tests were carried out at the same time each day to eliminate possible effects of the diurnal activity cycle of each bird.

Although the eyelids were held open for the duration of the session, spontaneous extensions of the nictitating membrane served to moisten the cornea. Additionally, at the termination of each session, two drops of normal saline were applied to the cornea. The body weight of the birds was monitored regularly and maintained within 10% of the body weight before headplating.

A test session commenced with restraining the owl and placing it in the headholder. The eyelids were cleaned with 95% ethanol and retracted. The pupillometer was placed in front of the right eye. The anechoic chamber lights were turned off, the doors to the chamber closed, and the bird allowed to adapt to the dark for 5 min before stimulus presentations began. Experiments were carried out in the dark to prevent the bird from acquiring visual cues of sound source locations. After each session, the tape was removed from the eyelids,

and adhesive residue, if any, was removed with 95% ethanol. The bird was then weighed and returned to its flight chamber.

Apparatus

Experiments were carried out in a sound-attenuating anechoic chamber (Industrial Acoustics) measuring 2.7 m in height, 3.9 m in width, and 4.5 m in length. It reduced noise by 65 dB at 500 Hz and had a free-field performance between 0.3 kHz and 10.0 kHz. Stimuli were presented from speakers (Alpine 6020AX) mounted on a 90° arc (71 cm diameter) centered on the owl's head at eye level, or, on a speaker-mount 1.98 m directly in front of the bird at eye level. Speaker position is expressed in degrees of azimuth (az) and elevation (el). Negative azimuths indicate sources to the left of midline and negative elevations indicate sources below eye-level.

The pupil was monitored using an infra-red (IR) pupillometer, consisting of an IR light-emitting diode (LED; F5D1QT; emission peak at 880 nm; QT Optoelectronics, Sunnyvale, Calif.) and an IR detector diode (QSC114; absorption maximum at 880 nm; QT Optoelectronics, Sunnyvale, Calif.). The detector and emitter were chosen for their small size (emitter: 6.4 mm high × 4.7 mm diameter; detector: 4.9 mm high × 3 mm diameter) and for their low output in the visible spectrum. The emitter, mounted 2.5 cm from the cornea, illuminated the whole cornea. The detector was mounted 5 mm from the eye and detected IR light reflected from the ventral-nasal quadrant of the eye. Output from the IR detector was amplified and digitized at 1000 samples s⁻¹ in the masked detection and frequency discrimination tasks, and at 1875 samples s⁻¹ in the spatial discrimination task (12-bit resolution).

During all sessions, the bird was additionally monitored using an IR-sensitive camera, the output of which was stored on video tape.

Auditory stimuli

Target stimuli consisted of tone bursts and band-limited noises, all of which were shaped with a 100-ms trapezoidal envelope with linear 5-ms rise and fall times. The A-weighted sound pressure level (SPL_A) of all stimuli was measured at a location corresponding to the center of a bird's head with a 1/2-inch microphone (Brüel and Kjør 4176) and sound-level meter (Brüel and Kjør 2625). The output from the SPL meter was delivered to a spectrum analyzer (Hewlett-Packard 35665A) to measure spectrum levels.

Carriers for tone bursts were generated by the spectrum analyzer, multiplied by the 100-ms trapezoidal envelope, attenuated with programmable attenuators (PA4, Tucker-Davis Technologies), amplified (HB6, Tucker-Davis Technologies), and delivered to the speakers.

Narrowband stimuli consisted of gammatone-filtered noises matched to the tuning curves of auditory nerve fibers of the owl (Köppl 1997). They were digitally generated offline, stored ('frozen'), and converted into analog format at 30,000 samples s⁻¹ (Modular Instruments M308, or Tucker-Davis Technologies Power DAC PD1).

Continuous broadband noise (56 dB SPL_A), used as a masker in the masked detection experiment, was generated by a custom-built noise generator and added to the narrowband signal after attenuation. The power spectrum of the masker measured after transduction by the speaker varied by less than 2.5 dB between 2 kHz and 12 kHz. The levels of the masker and stimuli were checked before each session.

Acquisition and analysis of pupillary response

Figure 1 shows the data acquisition scheme. A total of 5 s of the pupillometer output was digitized, thus acquiring output for 1 s before stimulus onset and 4 s after stimulus onset (stimuli were 100 ms long). Digitized traces were analyzed using Matlab 5.3 (The Mathworks).

The pupil, in the absence of any lighting except the IR source, was dilated to a large extent, but not to the fullest extent possible, due to the presence of small amounts of visible light in the IR source.

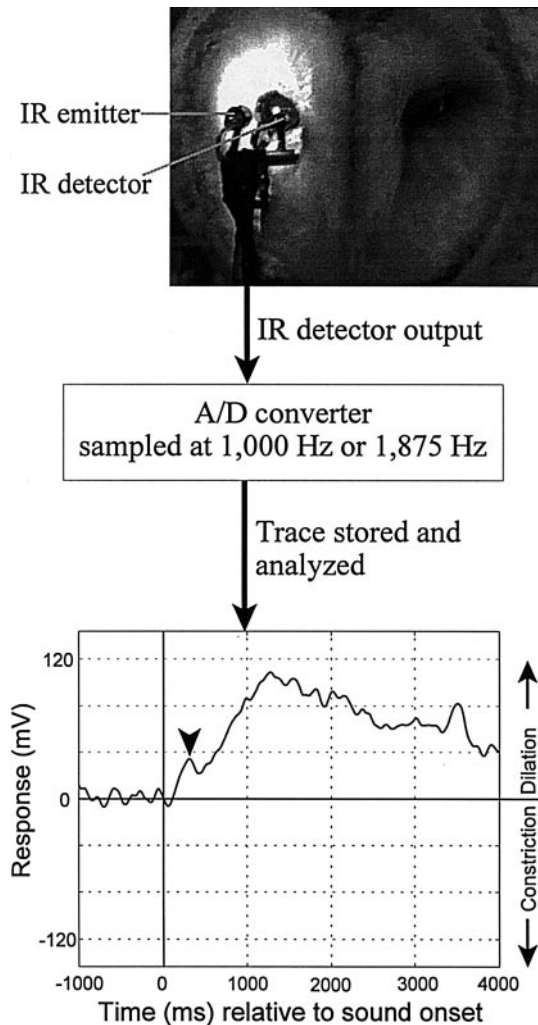


Fig. 1 Schematic representation of the data-acquisition procedure. The bottom panel shows a typical unhabituated pupillary dilation response (PDR) to an auditory stimulus (bird no. 882). The response trace shows an oscillation prior to the onset of the 6-kHz tone (-1000 ms to 0 ms). Shortly after sound onset (0 ms), the trace shows an upward deflection, corresponding to a dilation of the pupil. The dilation comprises two components: a fast transient component (*arrowhead*) and a sustained dilation

Reflectance values were expressed relative to the value at stimulus onset, yielding a response in millivolts. A dilation caused a brightening of the eye, presumably due to an increase in the light reflected from the retina, which in turn evoked an upward (positive) deflection in the trace. Conversely, a constriction caused a downward (negative) deflection of the response trace. Deflections below baseline were seldom seen, and when seen, were associated with the extension of the nictitating membrane across the cornea. All trials in which a nictitating membrane extension occurred within 3 s of trial onset (2 s after sound onset) were discarded.

Results

Acoustically-evoked pupillary response

A pupillary response evoked by the first presentation of a sound to an owl is shown in Fig. 1. In an acclimatized

bird, IR reflectance from the eye oscillated about a baseline value (Fig. 1, bottom panel; -1000 ms to 0 ms). Upon presentation of a tone from a speaker directly in front (0° az; 0° el; Fig. 1, 0 ms), the oscillation stopped, and the response as measured by the detector increased, accompanied by a clear pupillary dilation on the video monitor. The PDR typically consisted of three components: a fast transient dilation (*arrowhead*), a brief constriction, finally followed by a slow and prolonged dilation. Although all these components could be seen in all the birds tested, the precise shape of the response varied between birds, as the detector and emitter could not be positioned in precisely the same position for each bird. In Fig. 1 the response had not returned to baseline (level before stimulus delivery) 4 s after stimulus onset, indicating that the pupil was still dilated. The prolonged dilation lasted longer than 15 s in the case of very loud stimuli. The oscillatory behavior resumed long before the trace returned to baseline, even while the pupil was dilated. In Fig. 1 the oscillatory behavior resumed about 1.5 s after sound onset. The kinetics of the onset of the fast component of the PDR was relatively resistant to the amplitude of the stimulus, while the slow component was much more sensitive to parametric changes in the stimulus.

The latency of the response was determined by examining a mean trace, averaged over 150–200 trials. In such an averaged trace, the pre-stimulus oscillations in the response, being unrelated to the stimulus onset, are averaged out, emphasizing the fast component and making it easy to identify the response onset. The latency of the dilation response in individual trials cannot be determined with precision, as it is, in part, determined by the state of constriction of the pupil at the time of stimulus presentation.

The duration and magnitude of the PDR depended upon the detectability of a stimulus. The relationship between SNR and the dilation response was tested by presenting a gammatone target (centered at 4490 Hz) at varying levels in the presence of a continuous, constant-level, broadband masker. The masker was presented from the same speaker (0° az; $+5^\circ$ el) as the target. Each session consisted of 80–100 trials presented at intervals of 15 ± 3 s. The SNR was varied at random in 10-dB steps over a total range of 20 dB. Every trial with the target was followed by at least two catch trials in which no target was presented. Results are shown in Fig. 2a, in which response is plotted as a function of time relative to target onset. The PDR increased both in magnitude and duration (time over which the sustained rise persists). The magnitude of the response was quantified by measuring the area between the baseline and trace over the first 2 s after sound onset, a quantity that we term the “integrated response” (with units of millivolt-seconds). At high SNRs, the integrated response was large (black areas in Fig. 2a), but when there was no target, or when the target was presented at a sub-threshold level, the integrated response showed only small departures from the baseline. The areas under the trace in the 1-s segment before

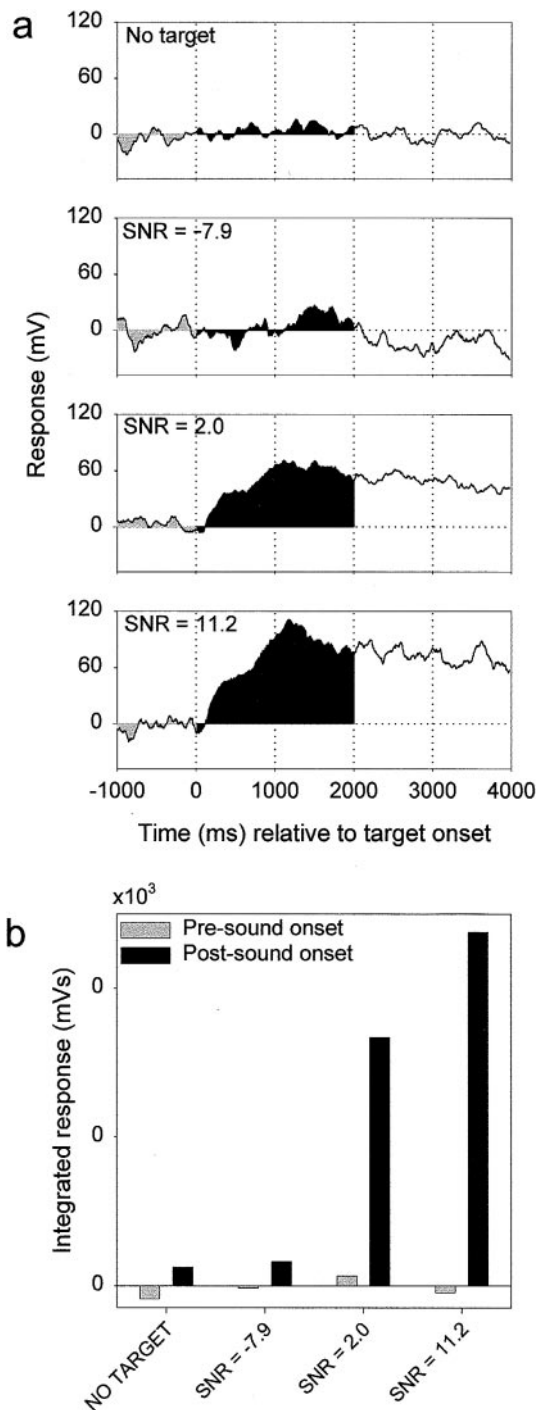


Fig. 2a, b Stimulus-response relationship. **a** Averaged traces from trials at varying signal-to-noise ratios (SNRs). Each panel shows the mean of 20 traces from trials in which a gammatone target was presented at different levels in the presence of a continuous, constant-level masker from the same speaker. The SNR in each set of trials is shown to the upper left. The gray and black shaded areas indicate the periods over which each trace was integrated to derive, respectively, areas for the 1-s pre- and 2-s post-target periods. **b** Integrated responses (area in the 2-s post-target period) are plotted as a function of SNR. Note that the pre-target traces have relatively little departure from the baseline, while the post-target traces show a positive integrated response, in concord with the upward deflection of the traces in **a** (bird no. 880)

stimulus onset (gray areas in Fig. 2a) were small and could be negative or positive. By contrast, post-stimulus integrated responses were always positive, and increased with the level of the target. The areas shaded in Fig. 2a are plotted as a function of SNR in Fig. 2b for the periods before and after stimulus-onset.

Habituation and recovery of the PDR

The PDR in the barn owl habituated when a stimulus was presented repeatedly. Habituation of the PDR to the first four presentations of a pure tone (6000 Hz, 100 ms, 54 dB SPL_A; presented every 10 ± 2 s) in a single session is shown in Fig. 3a. The decrement in response consisted of a decrease in both the magnitude and duration of the response. The fast component of the dilation (arrows) persisted, while the slow component attenuated rapidly. Habituation within a few presentations was typical when the sound stimulus was a pure tone. The integrated responses computed from the first 25 trials of the session, including the traces in Fig. 3a, are plotted as a function of trial number in Fig. 3b (circles). Note that the mean integrated response of habituated trials remained above zero as the fast component does not habituate.

The number of trials required to reach a stable habituated value was related to the level of the stimulus: quieter stimuli elicited smaller PDRs that habituated in fewer trials, while louder stimuli elicited larger PDRs that habituated over a larger number of trials. Similarly, habituation was also dependent on the bandwidth of the stimulus: narrowband noises at an SPL equal to that of a pure tone were much more resistant to habituation. Figure 4 shows the integrated responses from all trials in the session, the first 25 trials of which were shown in Fig. 3. The scatter of the habituated response late in the session was the same as in the beginning of the session. If the mean integrated response changed over the course of a session (t -test, $P > 0.05$), or if the variance of a particular session was different from that of the other sessions with the same animal (F -test, $P > 0.05$), data from that session were rejected.

The PDR, habituated by the repeated presentation of an auditory stimulus, recovered upon the presentation of a novel stimulus. Figure 4 shows that the PDR, habituated to 6 kHz, recovered upon presentation of a tone at 7 kHz (filled square). The recovered response was always smaller than the response to the first presentation of sound in the session. Recovery of the response by a novel sound demonstrates that the habituation is not due to muscle fatigue. The ability to recover a habituated response with a novel stimulus was used to assess frequency discrimination and the MAA.

Frequency discrimination

To study frequency discrimination, the PDR was allowed to habituate by repeated presentation of a

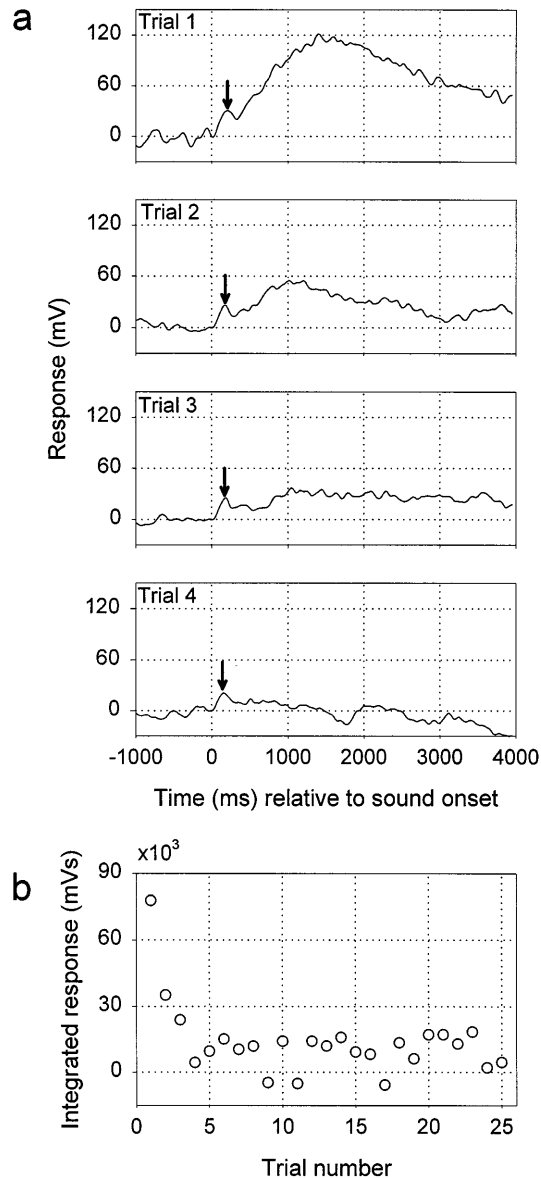


Fig. 3 **a** Response traces from four consecutive presentations of a 100-ms, 6-kHz tone (bird no. 882). The response habituates significantly over the course of these four trials. The fast component of the dilation (*arrows*) does not habituate to the same extent as does the slow component over these trials. **b** The integrated response of the first 25 trials in the session. Note that the integrated response falls rapidly over the first few trials to a stable mean level

reference tone of either 3 kHz (49 dB SPL_A) or 6 kHz (53 dB SPL_A) at intervals of 10 ± 2 s. Every tenth trial was designated a “test trial”, in which a tone that differed from the reference tone by 0 Hz, 125 Hz, 250 Hz, 500 Hz, or 1000 Hz was presented at the same SPL as the reference tone. Note that the 0-Hz test trial was actually a catch trial in which the reference tone was presented in the place of a tone at a different frequency. Catch trials constituted one-fourth of all test stimuli. Each session comprised a total of 160–200 trials.

Figure 5 shows the method by which these data were analyzed. Response traces from four test trials (reference

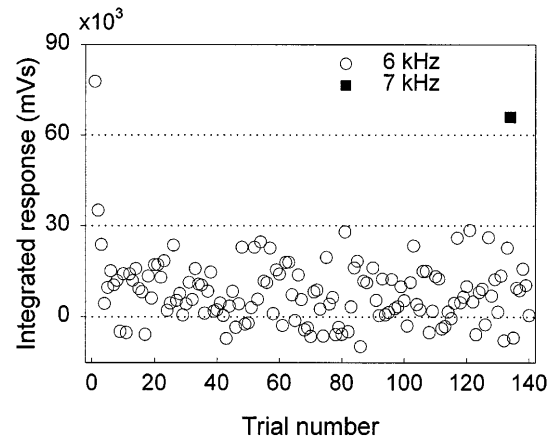


Fig. 4 Recovery of habituated PDRs by a novel stimulus. Data shown here are from the same session as in Fig. 3. Responses habituated by repeated presentation of a 6-kHz tone (*open circles*), recover upon presentation of a 7-kHz tone (*filled square*). There is no significant change in either the mean or the variance of the response of the habituated response over the course of the session

frequency + 500 Hz; solid lines) and four catch trials (dashed lines) are shown in Fig. 5a. Before tone onset (–1000 ms to 0 ms), there was no systematic difference between the traces from test and catch trials. Upon stimulus presentation, however, the trajectory of the traces diverged. The traces from the test trials showed a typical, two-phase, upward deflection lasting more than 2 s in this case, whereas the catch-trial traces remained closer to the baseline. The integrated responses for all trials in this session are shown in Fig. 5b, with the test trials and catch trials shown in Fig. 5a labeled with squares and triangles, respectively. In addition, integrated responses from test trials with a tone 250 Hz greater than the reference tone are shown (filled diamonds).

Figure 5b shows that repeated presentation of the reference tone caused habituation within the first ten trials. The habituated response showed a scatter about a mean level, which was computed by averaging the integrated responses from trial number 11 onwards. The dispersion of the habituated response about the mean habituated response is highlighted by solid horizontal lines that show the mean \pm 2SD. We quantified the departure of each test trial from the mean habituated response in terms of the standard deviation, thus deriving a normalized score (Green and Swetts 1966). This score expresses the difference of the response to a novel tone relative to the large number of responses to the reference tone, and is therefore a way of normalizing data gathered from different animals and over many sessions. Catch trials were distributed about the mean of the habituated responses, while the +250-Hz and +500-Hz test trials were distributed around higher normalized scores. Such data were gathered from 40 sessions, yielding a distribution of normalized scores for catch trials and for each of the test trials. A one-tailed *t*-test was then used to assess the likelihood that the test and

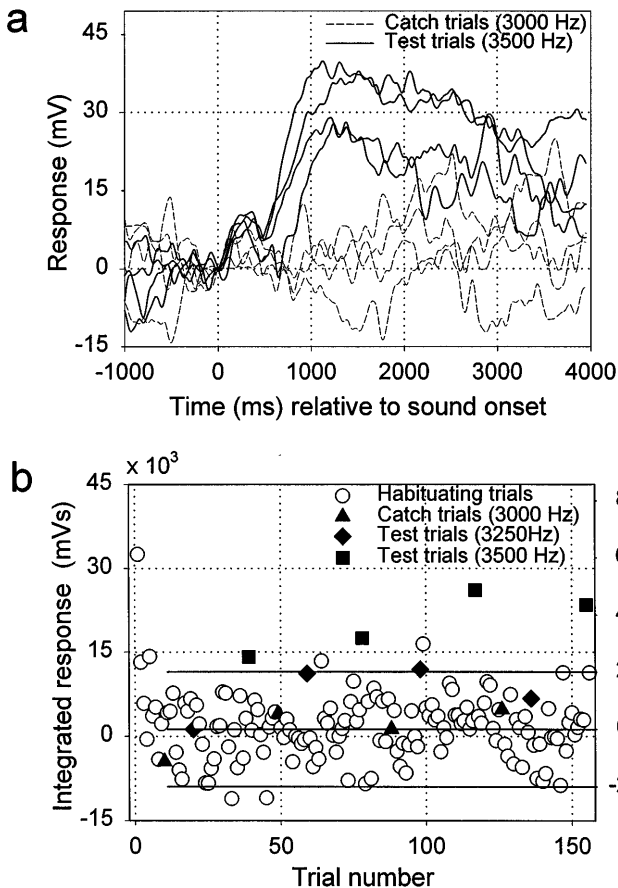


Fig. 5a, b Statistical analysis of PDRs. The dilation responses to habituating and test stimuli during one frequency-discrimination session (bird no. 883). **a** Traces of PDR to catch trials (3000-Hz tone) and test trials (3500-Hz tone). After sound onset, there is a significant dilation to test trials tone but not to catch trials. **b** Integrated responses plotted as a function of trial number. Integrated responses show rapid decrease to a habituated level over the first few trials. The PDR recovers to a larger extent if a test tone differs from the reference tone by 500 Hz (filled squares) than by 250 Hz (filled diamonds). Note also that all trials at the larger difference cause recovery, while only two trials at the 250-Hz difference cause significant recovery of the habituated response. Axis labels on the right represent the normalized scores that express departure of the catch and test trials from the mean of all the habituating trials (unfilled circles)

catch trials were drawn from the same population at a criterion of $P = 0.005$.

The results of the frequency discrimination experiment are shown in Fig. 6, in which the normalized scores are plotted as a function of the difference in the frequencies of the reference and test tones (Δf) at reference tones of 3000 Hz (Fig. 6a) and 6000 Hz (Fig. 6b). Frequencies at which test responses were discriminable from catch-trial responses are indicated by asterisks. The frequency discrimination function was asymmetric. The owl was better able to distinguish frequencies that were higher than the reference as compared to those that were lower. For example, the birds discriminated a Δf of +125 Hz (re: 3 kHz) but not a Δf of -125 Hz. At a reference frequency of 6 kHz, the minimal Δf the owl could detect was +250 Hz, as opposed to the +125-Hz

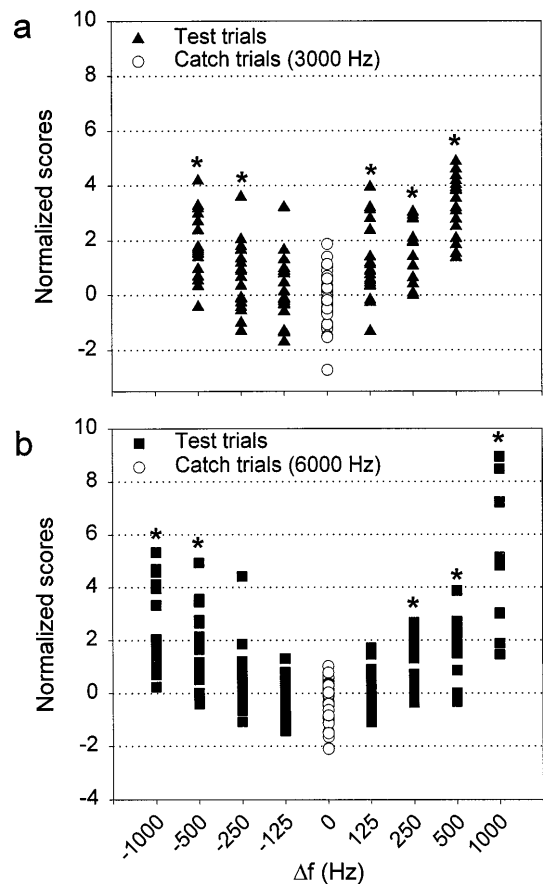


Fig. 6a, b Frequency discrimination using the habituation-recovery paradigm. **a** Differences in the responses (normalized scores) to test (triangles) and reference tones (3 kHz; circles) are plotted as a function of the difference in the frequencies of the tones. Test frequencies at which the responses are different from those to the catch (one-tailed t -test, $P = 0.005$) are indicated by asterisks. **b** Differences in response to test (squares) and reference tones (6 kHz; circles). As in **a**, asterisks denote a significant difference at the criterion level ($P = 0.005$) using the one-tailed t -test. Data were pooled from two birds after determining that there were no systematic variations across sessions or across animals

difference detected at 3 kHz (Fig. 5). At 6 kHz too, higher test frequencies (re: reference tone) were more easily discriminated than were lower test frequencies.

Spatial discrimination

To study spatial discrimination, the PDR was habituated to a sound from a speaker at one location and was examined for recovery when the same sound was presented from another speaker at a different azimuth. The two speakers were evenly spaced on either side of the midline at eye-level separated by 1.5°, 3°, 4.5°, 6°, or 14.5°. A distance of 1.98 m from the center of the bird's head was necessary to achieve the small angular separations given the diameter of the loudspeakers. This distance, which was more than 2.5 times longer than that used in the frequency-discrimination task, correspondingly increased the latency of the response relative to the

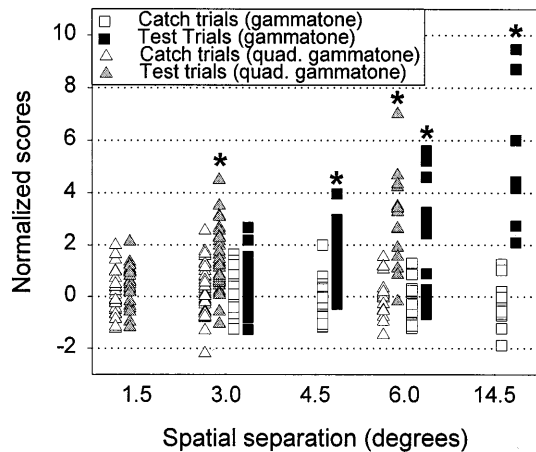


Fig. 7 Discrimination of sound-source azimuth. Differences (normalized scores) in the responses to sounds (single gammatone – squares; quadruple gammatone – triangles) from test (filled symbols) and reference (unfilled symbols) locations are plotted as a function of the azimuthal separation of the two locations. The reference location is to the right of midline (at eye level), while the test location is to the left of midline. Locations at which the responses to test and catch trials are significantly different (one-tailed *t*-test, significance level $P = 0.005$) are marked by asterisks. Data were pooled from three birds

onset of the sound at the speaker. In a given session, only one spatial separation was tested; the speakers were moved between sessions in order to test a different spatial separation. Each session comprised 180–250 trials, at intervals of 11 ± 2 s. Test trials occurred once every 40 trials; the sound was presented from the habituating location in all other trials. Of the 39 trials that separated any two test trials, the intermediate (20 trials after any test) was designated as a catch trial, to which test trials were compared. Tests were conducted with either a frozen gammatone noise centered at 4490 Hz or a frozen noise consisting of four gammatones centered at 4 kHz, 5 kHz, 6 kHz, and 7 kHz summed together (quadruple gammatones). As described above, sound output from both speakers was monitored before each session to ensure that the amplitude spectra of the sounds were matched within 0.5 dB.

Results are shown in Fig. 7 for both the narrowband (single gammatone; squares) and wider-band (quadruple gammatone; triangles) stimuli. Integrated responses of test trials were expressed as normalized scores, as in the frequency discrimination experiment, and plotted as a function of speaker separation. As Fig. 7 shows, a change in the location of the source recovered the habituated PDR, and the magnitude of the recovered response was larger for larger angular separations. For the narrowband signal (black squares), at angular separations of 14.5°, 6°, or 4.5°, the magnitude of PDR was significantly different (one tailed *t*-test; $P = 0.005$) from responses to catch trials (circles). When the angular separation was decreased to 3°, the responses to catch and test trials were indistinguishable. Thus, using this paradigm and a narrowband noise, the horizontal MAA is 4.5°. Figure 7 suggests that with a wider-band

stimulus (gray triangles) spatial resolution is better. The birds were clearly able to discriminate the 3° separation, but failed when the angular separation was reduced to 1.5°. The horizontal MAA using a quadruple gammatone as the probe is therefore about 3E.

Discussion

We have described an acoustically-evoked pupillary dilation response in the barn owl, and some of the conditions under which it is evoked, habituated, and recovered. This response is elicited in the unanesthetized, head-restrained owl by an auditory signal presented in quiet, the response magnitude increasing with increasing SNR. Repeated presentation of a stimulus habituates the PDR, but a change in stimulus frequency or location recovers it. The latter phenomenon was used to examine frequency discrimination and to measure, for the first time, the MAA in the owl. Although auditory discrimination using a physiological component of the orienting response has been described earlier (see Introduction), the present study is the first to apply it to a study of auditory spatial discrimination.

The PDR offers a number of advantages to other methods of gathering behavioral discriminability data. Firstly, it can be readily evoked without training provided that the bird is acclimatized to restraint. Secondly, it does not require aversive stimuli as is often the case in classical conditioning. Thirdly, it can be evoked in a head-restrained preparation and is thus compatible with simultaneous electrophysiological recordings. This is an advantage shared with classical conditioning techniques (e.g., Oleson et al. 1973; Weinberger et al. 1973, 1975; Berger et al. 1976; Batra et al. 1989; Fitzpatrick et al. 1995), and is particularly important in the bird, in which eye-movements or pointing and reaching either do not exist or involve movements that would make it difficult to maintain stable recordings. These advantages of the PDR are true of other orienting reflexes such as the galvanic skin response or heart and respiration rates. The PDR, however, occurs over a faster time scale, and its timing can therefore be measured with greater precision.

There are, however, some limitations. The motivation and attentional state of the bird are not well defined. An animal performing an operant task is presumably motivated and attending in order to gain a reward or avoid aversive stimuli. The bird in the present paradigm has no such motive. It is therefore important that discrimination data obtained by all methods be compared before drawing general conclusions.

Another limitation is that a given test stimulus cannot be presented too often lest the bird habituate to that test stimulus. Typically, we presented only five or six in a session, but sessions with up to ten repetitions of the same stimulus yielded reliable results. Although the number of test trials is best kept low, the number of habituating trials is large, and their variance can be calculated with precision. This, in turn, allows us to

apply signal-detection theory (Green and Swetts 1966) to assess the significance of the response magnitude of individual trials relative to this control population. The signal detection approach also allows us to pool data across birds and sessions, provided that the variance of the responses in habituating trials is carefully monitored for constancy.

Neural pathways mediating the acoustically evoked PDR

Figure 8 shows the averaged traces from test and catch trials in the spatial discrimination task. Expansion of the time scale (Fig. 8b) shows that in both test and catch trials, the pupil first shows signs of dilation at about 30 ms after sound onset (25 ms after sound reaches the bird). About 110 ms after sound onset, the catch-trial trace peaks, while the test-trial trace continues to climb. The PDR thus shows components consistent with detection and discrimination. As the acoustical delay from speaker to head is about 5 ms, we can infer that detection and discrimination require, respectively, about 25 ms and 105 ms of processing time. This, of course, includes the time in the motor pathways, up to the beginning of activity in the dilator muscle of the iris.

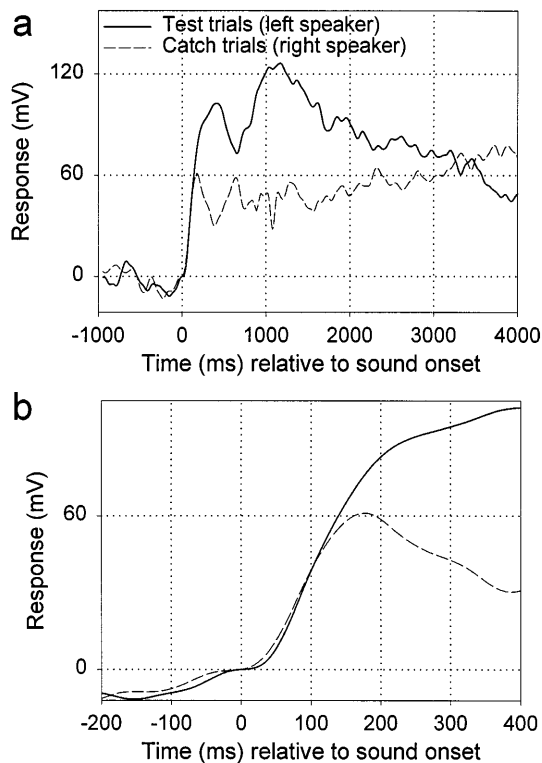


Fig. 8 Time-courses of habituated and recovered PDRs at two different time scales (**a**, **b**). Mean traces from test trials ($n = 12$) and catch trials ($n = 11$) in the spatial discrimination task from two sessions of bird no. 882 (speaker separation 4.5° ; single gammatone stimulus). On sound delivery, the two traces follow the same trajectory for the first 110 ms, after which the two traces diverge, yielding the time point at which we can observe discrimination

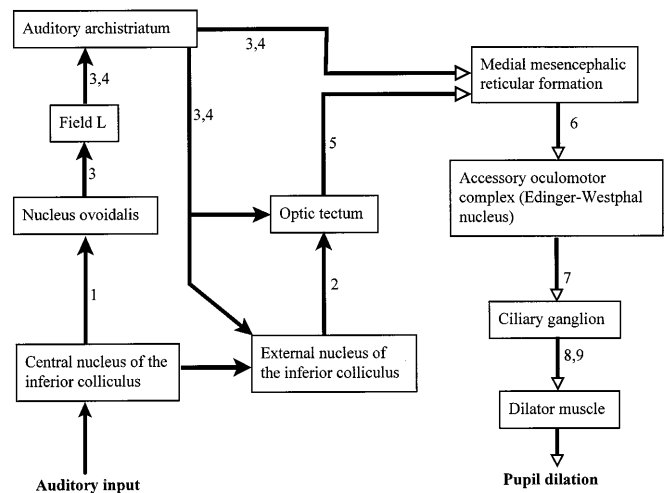


Fig. 9 A possible neural circuit connecting the auditory nuclei to the pupil. Citations for various pathways in the circuit are as follows: (1) Proctor and Konishi (1997), (2) Knudsen and Knudsen (1983), (3) Cohen et al. (1998), (4) Knudsen et al. (1995), (5) Masino and Knudsen (1992), (6) Gamlin and Reiner (1991), (7) Narayanan and Narayanan (1976), (8) Pilar et al. (1980), (9) Pilar et al. (1987)

An anatomical pathway that could subserve the acoustically evoked PDR is shown in Fig. 9. In the barn owl, the auditory archistriatum and the optic tectum send efferents to the medial mesencephalic reticular formation (MRF; Masino and Knudsen 1992; Knudsen et al. 1995). Tracing studies in the pigeon show that the MRF projects to the accessory oculomotor complex, the avian homolog of the Edinger-Westphal nucleus (Gamlin and Reiner 1991), which, in turn, projects to the ciliary ganglion (Narayanan and Narayanan 1976). A small subdivision of branch II of the ciliary nerve innervates the dilator muscle (Pilar et al. 1980, 1987). It is difficult to place precise constraints on where detection and discrimination might be occurring in these pathways based solely on data like that in Fig. 8. However, since the median latency in the auditory archistriatum is about 25 ms (Cohen and Knudsen 1995), it seems unlikely that the detection component is mediated through the telencephalic pathway shown. By contrast, discrimination takes up to 80 ms longer, and could well be mediated by the telencephalic pathway. The possible involvement of the auditory archistriatum in the present behavior is interesting because this part of the brain has been implicated as the site of working memory (Knudsen and Knudsen 1996). Working memory would probably be essential in a habituation-recovery paradigm such as ours, where the perception of a novel stimulus as different would necessitate a retention of parameters of the habituating stimulus presented roughly 10 s earlier.

Comparisons with earlier studies of auditory discrimination

Although the barn owl is an established animal model for the study of spatial hearing, its ability to discriminate

the difference in sound-source location, namely, MAA, has never been measured directly. Sound localization accuracy has instead been inferred from the accuracy of the owl's talon strikes (Konishi 1973) or head turns (Knudsen et al. 1979). Measuring head turns to targets within 10° of the midline, Knudsen et al. (1979) demonstrated azimuthal localization errors of 1.6° for one animal and 2.3° for the other (see their Table 3). If we assume that these errors represent the width of the neural image of the source, targets would have to be separated by 4.5° and 6.5°, respectively, to be discriminable at a criterion level of $P = 0.05$ (2 SDs). The current paradigm, despite the use of more stringent statistical criteria ($P = 0.005$), revealed finer resolution. The 3° MAA of the owl measured by our paradigm is thus comparable to that of the human listener (Blauert 1997; Table 2.1, p 39), but considerably smaller than that of other mammals with heads of similar sizes (Kavanagh and Kelly 1986, 1988; Heffner et al. 1994).

Our results also showed that the MAA was finer for broader-band stimuli, an observation consistent with the study of Knudsen and Konishi (1979) who compared the accuracy of head turns to targets emitting broadband noise or tones. It is also consistent with behavioral observations in humans showing that localization is facilitated by an increase in bandwidth (Chandler and Grantham 1992).

Frequency discrimination was investigated in owls trained to respond if the frequency of a tone differed from that of a memorized reference by Quine and Konishi (1974). Their results are comparable to ours for similar reference frequencies, except that one of their birds could discriminate a 25-Hz difference at 3500 Hz contrasted to the 125-Hz difference that we observed at 3000 Hz. This could reflect individual differences or a difference in testing paradigms. Compared to broadband or band-limited noises, pure tones are not very effective at eliciting the PDR, yielding habituated and recovery responses of a smaller magnitude, and the habituation-recovery paradigm may therefore not be the best method for assessing pure-tone discrimination.

Our results suggest that the PDR can also be used to assess masked detection thresholds although we did not study it in detail. Masked detection has been examined more thoroughly in the barn owl by Dyson et al. (1998), who used pure-tone targets. As we used gammatones as targets, our results are not directly comparable.

Acknowledgements This work was supported by grants from the National Institute Deafness and Communication Disorders (DC03925) and the McKnight Foundation for Neuroscience. Drs. Jagdeep Bala, Kip Keller, Matthew Spitzer, and Norman Weinberger provided us with many helpful suggestions and insights. Dr. Keller also provided technical assistance with hardware and software. David S. Nash assisted in some of the experiments. All experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee of the University of Oregon.

References

- Bagshaw MH, Benzie S (1968) Multiple measures of the orienting reaction and their dissociation after amygdectomy in monkeys. *Exp Neurol* 20: 175–187
- Bagshaw MH, Kimble DP, Pribram KH (1965) The GSR of monkeys during orienting and habituation and after ablation of the amygdala, hippocampus and infero-temporal cortex. *Neuropsychologia* 3: 111–119
- Batra R, Kuwada S, Stanford TR (1989) Temporal coding of envelopes and their interaural delays in the inferior colliculus of the unanesthetized rabbit. *J Neurophysiol* 61: 257–268
- Berger TW, Alger B, Thompson RF (1976) Neuronal substrate of classical conditioning in the hippocampus. *Science* 192: 483–485
- Blauert J (1997) Spatial hearing. The psychophysics of human sound localization. MIT Press, Cambridge
- Chandler DW, Grantham DW (1992) Minimum audible movement angle in the horizontal plane as a function of stimulus frequency and bandwidth, source azimuth, and velocity. *J Acoust Soc Am* 91: 1624–1636
- Cohen YE, Knudsen EI (1995) Binaural tuning of auditory units in the forebrain archistriatal gaze fields of the barn owl: local organization but no space map. *J Neurosci* 15: 5152–5168
- Cohen YE, Miller GL, Knudsen EI (1998) Forebrain pathway for auditory space processing in the barn owl. *J Neurophysiol* 79: 891–902
- Dyson ML, Klump GM, Gauger B (1998) Absolute hearing thresholds and critical masking ratios in the European barn owl: a comparison with other owls. *J Comp Physiol A* 18: 695–702
- Fitzpatrick DC, Kuwada S, Batra R, Trahiotis C (1995) Neural responses to simple simulated echoes in the auditory brain stem of the unanesthetized rabbit. *J Neurophysiol* 74: 2469–2486
- Gamlin PD, Reiner A (1991) The Edinger-Westphal nucleus: sources of input influencing accommodation, pupilloconstriction, and choroidal blood flow. *J Comp Neurol* 306: 425–438
- Graham FK (1973) Habituation and dishabituation of responses innervated by the autonomic nervous system. In: Peeke HVS, Herz MJ (eds) *Habituation*, vol I. Academic Press, New York
- Green DM, Swets JA (1966) Signal detection theory and psychophysics. Wiley, New York
- Hare RD (1968) Psychopathy, autonomic functioning, and the orienting response. *J Abnorm Psychol* 73: 1–24
- Heffner RS, Heffner HE, Kearns D, Vogel J, Kooy G (1994) Sound localization in chinchillas. I. Left/right discriminations. *Hear Res* 80: 247–257
- Jay MF, Sparks DL (1987) Sensorimotor integration in the primate superior colliculus. I. Motor convergence. *J Neurophysiol* 57: 22–34
- Kavanagh GL, Kelly JB (1986) Midline and lateral field sound localization in the albino rat (*Rattus norvegicus*). *Behav Neurosci* 100: 200–205
- Kavanagh GL, Kelly JB (1988) Hearing in the ferret (*Mustela putorius*): effects of primary auditory cortical lesions on thresholds for pure tone detection. *J Neurophysiol* 60: 879–888
- Knudsen EI, Knudsen PF (1983) Space-mapped auditory projections from the inferior colliculus to the optic tectum in the barn owl (*Tyto alba*). *J Comp Neurol* 218: 187–196
- Knudsen EI, Knudsen PF (1996) Disruption of auditory spatial working memory by inactivation of the forebrain archistriatum in barn owls. *Nature (Lond)* 383: 428–431
- Knudsen EI, Konishi M (1979) Mechanisms of sound localization by the barn owl (*Tyto alba*). *J Comp Physiol A* 133: 13–21
- Knudsen EI, Blasdel GG, Konishi M (1979) Sound localization by the barn owl (*Tyto alba*) measured with the search coil technique. *J Comp Physiol A* 133: 1–11
- Knudsen EI, Cohen YE, Masino T (1995) Characterization of a forebrain gaze field in the archistriatum of the barn owl: microstimulation and anatomical connections. *J Neurosci* 15: 5139–5151

- Konishi M (1973) Locatable and nonlocatable acoustic signals for barn owls. *Am Nat* 107: 775–785
- Köppel C (1997) Frequency tuning and spontaneous activity in the auditory nerve and cochlear nucleus magnocellularis of the barn owl *Tyto alba*. *J Neurophysiol* 77: 364–377
- Korn JH, Moyer KE (1968) Effects of set and sex on the electrodermal orienting response. *Psychophysiology* 4: 453–459
- Maher TF, Furedy JJ (1979) A comparison of the pupillary and electrodermal components of the orienting reflex in sensitivity to initial stimulus presentation, repetition and change. In: Kimmel HD, Olst EH van, Orlebeke JF (eds) *The orienting reflex in humans*. Lawrence Erlbaum Associates, New York
- Masino T, Knudsen EI (1992) Anatomical pathways from the optic tectum to the spinal cord subserving orienting movements in the barn owl. *Exp Brain Res* 92: 194–208
- Narayanan CH, Narayanan Y (1976) An experimental inquiry into the central source of preganglionic fibers to the chick ciliary ganglion. *J Comp Neurol* 166: 101–109
- Oleson TD, Westenberg IS, Weinberger NM (1972) Characteristics of the pupillary dilation response during Pavlovian conditioning in paralyzed cats. *Behav Biol* 7: 829–840
- Oleson TD, Vododnick DS, Weinberger NM (1973) Pupillary inhibition of delay during Pavlovian conditioning of paralyzed cats. *Behav Biol* 8: 337–346
- Olst EH van, Heemstra ML, Kortenaar T ten (1979) Stimulus significance and the orienting reaction. In: Kimmel HD, Olst EH van, Orlebeke JF (eds) *The orienting reflex in humans*. Lawrence Erlbaum Associates, New York
- Payne RS (1971) Acoustic location of prey by barn owls (*Tyto alba*). *J Exp Biol* 54: 535–573
- Pilar G, Landmesser L, Burstein L (1980) Competition for survival among developing ciliary ganglion cells. *J Neurophysiol* 43: 233–254
- Pilar G, Nunez R, McLennan IS, Meriney SD (1987) Muscarinic and nicotinic synaptic activation of the developing chicken iris. *J Neurosci* 7: 3813–3826
- Proctor L, Konishi M (1997) Representation of sound localization cues in the auditory thalamus of the barn owl. *Proc Natl Acad Sci USA* 94: 10421–10425
- Quine DB, Konishi M (1974) Absolute frequency discrimination in the barn owl. *J Comp Physiol* 93: 347–360
- Sokolov EN (1963) Higher nervous functions: the orienting reflex. *Annu Rev Physiol* 25: 545–580
- Takahashi TT, Keller CH (1994) Representation of multiple sound sources in the owl's auditory space map. *J Neurosci* 14: 4780–4793
- Weinberger NM, Oleson TD, Haste D (1973) Inhibitory control of the conditional pupillary dilation response in the paralyzed cat. *Behav Biol* 9: 307–316
- Weinberger NM, Oleson TD, Ashe JH (1975) Sensory system neural activity during habituation of the pupillary orienting reflex. *Behav Biol* 15: 283–301
- Weisbard C, Graham PK (1971) Heart-rate change as a component of the orienting response in monkeys. *J Comp Physiol Psychol* 76: 74–83