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ORIGINAL PAPER

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Orienting responses and vocalizations produced by microstimulation in the superior colliculus of the echolocating bat, *Eptesicus fuscus*

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Abstract An echolocating bat actively controls the spatial acoustic information that drives its behavior by directing its head and ears and by modulating the spectro-temporal structure of its outgoing sonar emissions. The superior colliculus may function in the coordination of these orienting components of the bat's echolocation system. To test this hypothesis, chemical and electrical microstimulation experiments were carried out in the superior colliculus of the echolocating bat, *Eptesicus fuscus*, a species that uses frequency modulated sonar signals. Microstimulation elicited pinna and head movements, similar to those reported in other vertebrate species, and the direction of the evoked behaviors corresponded to the site of stimulation, yielding a map of orienting movements in the superior colliculus. Microstimulation of the bat superior colliculus also elicited sonar vocalizations, a motor behavior specific to the bat's acoustic orientation by echolocation. Electrical stimulation of the adjacent periaqueductal gray, shown to be involved in vocal production in other mammalian species, elicited vocal signals resembling acoustic communication calls of *E. fuscus*. The control of vocal signals in the bat is an integral part of its acoustic orienting system, and our findings suggest that the superior colliculus supports diverse and species-relevant sensorimotor behaviors, including those used for echolocation.

Keywords Superior colliculus · Echolocating bat · Acoustic orientation · Microstimulation · Sensorimotor integration

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Abbreviations *CF* constant frequency · *FM* frequency modulated · *IPI* interpulse interval · *KA* kainic acid · *MRF* midbrain reticular formation · *PAG* periaqueductal grey · *SC* superior colliculus · *SLN* superior laryngeal nerve

Introduction

The midbrain superior colliculus (SC; optic tectum) functions as a sensorimotor interface, linking sensory-based spatial information to orienting responses. SC-mediated orientation responses are rapid, directed, saccadic movements of the eyes, ears, head, and body, and occur following the detection of a target, which may be visual, auditory, tactile, or electric. A representation of sensory space and computational signals for producing complex, coordinated, and purposeful motor responses are thought to be encoded in the SC. Indeed, orienting movements of the eyes, ears, head, and body have been evoked by microstimulation of the deep SC in numerous vertebrate species, and the map of movements follows a topographic scheme that corresponds to the sensory representation (Robinson 1972; Stein and Meredith 1993).

The particular repertoire of elicited motor responses reflects the use of each sensory modality in an animal's orientation behavior and its ability to respond with a movement that brings a target stimulus on-axis. For example, microstimulation of the primate SC produces predominantly oculomotor behavior, with the location of the stimulating electrode corresponding to the metrics of eye and head movement responses (e.g., Cowie and Robinson 1994; Freedman et al. 1996; Stanford et al. 1996). The contribution of eye and head movement components pairs well with the range of the primate's visual field, and target-directed gaze prepares the animal for visually guided reaching and grasping of visual and acoustic targets. In the barn owl, whose eyes and external ears are immobile, stimulation elicits head movements that bring the eye and ear sensory axes

in-line with the target of interest (DuLac and Knudsen 1990). In the cat, for which both hearing and vision are important to orienting behavior, movements of the external ears, or pinnae, are evoked along with eye and head movements (Stein and Clamann 1981).

For the echolocating bat, an animal that navigates through space and forages in darkness using a sonar orientation system, the coordinated activity of spatial information processing, vocal-motor, and head-aim systems is required. Schuller and Radtke-Schuller (1988, 1990) elicited sonar vocalizations with electrical microstimulation of the deep SC of the horseshoe bat, suggesting a link between the production of sonar vocalizations and the orienting motor function of the SC. Anatomical data show reciprocal connections between the SC and the paralemniscal tegmental area (Metzner 1996; Sinha et al. 2000), a region implicated in echolocation behavior in the rufous horseshoe bat, *Rhinolophus rouxi* (Metzner 1989, 1993; but see also Pillat and Schuller 1998). However, electrically elicited movements of the head and pinna have not been systematically studied in the SC of the bat, leaving open the question of whether this midbrain structure plays a role in the coordinated activity of spatial-acoustic, vocal-motor, and pinna-/head-orienting components for sonar orientation.

Observations from the field and the laboratory indicate that bats' sonar orientation systems are directional (Griffin 1958; Masters et al. 1985; Simmons 1987; Hartley and Suthers 1989; Valentine and Moss 1998). Once a sonar target is detected, the bat aims its head such that its echolocation sounds are directed at the target like an acoustic flashlight. The ensonified target returns echoes that are received through the bat's external ears. The bat's ear is an intricate structure of cartilage, skin, and muscle, and its complex structural morphology produces spectral transformations of incoming sound waves for vertical sound localization (Lawrence and Simmons 1982; Jen and Chen 1988; Obrist et al. 1993; Wotton et al. 1995, 1996). Using spectral, temporal, and amplitude features of complex echoes, the auditory system of the bat extracts and processes information to assign the horizontal (Shimozawa et al. 1974; Simmons et al. 1983) and vertical (Grinnell and Grinnell 1965; Lawrence and Simmons 1982; Wotton et al. 1995) location of a stimulus. Target range is thought to be derived from the time delay between an outgoing sonar vocalization and its returning echo (Hartridge 1945; Simmons 1973). These spatial localization cues that describe a sonar target's position in three-dimensions – azimuth, elevation, and distance – must then be integrated with motor-based signals in order to guide behavior – head and pinna movements and sonar vocalizations – appropriate for the tasks of detecting, tracking, and capturing insect prey. The coordination of sensory information with motor output would allow the bat to regulate actively the reception of additional sensory input, and to modulate the acoustic information it uses for different sonar tasks. Based on studies of the cat SC, in which microstimulation elicited head and pinna movements (Harris 1980; Stein and Clamann 1981), and the horseshoe bat SC, in which stimulation evoked species-typical sonar vocalizations containing both constant frequency (CF) and frequency-modulated (FM) components (Schuller and Radtke-Schuller 1988, 1990), we conducted a series of experiments designed to broaden cross-species comparisons of SC function and to detail the coordination of orienting behaviors elicited by SC stimulation. In particular, we used microstimulation techniques to study the SC functional organization in *Eptesicus fuscus*, a bat that uses FM echolocation signals for acoustic orientation and has been the subject of extensive behavioral research (for review see Moss and Schnitzler 1995). These experiments focused not on a single orienting behavior elicited by microstimulation of the bat SC, but instead on a complex of behaviors that operate together to support acoustic orientation by sonar.

Materials and methods

Animal subjects

Big brown bats (*E. fuscus*) were caught wild in eastern Massachusetts and Maryland, and were housed in the laboratory in small groups in cages or in a larger enclosure where they could fly freely. The light-dark schedule was adjusted to produce active nocturnal conditions during the day. The temperature and humidity in the bat colony area were maintained at 25°C and 50%, respectively. Bats were fed *ad libitum* a diet consisting of nutritionally supplemented mealworms (*Tenebrio molitor*) and vitamin-supplemented water.

Surgical procedure, electrode implantation, and topographical mapping

Prior to an experiment, each bat underwent a surgical procedure to expose the SC and to secure a mounting pedestal as described previously (Valentine and Moss 1997; see also Suga et al. 1983; Suga and Horikawa 1986). After recovering for at least 48 h from the preparatory surgery, the bat was positioned in a double-walled acoustic chamber with its external ears visible in the field of a video camera mounted above the bat (Fig. 1). In most SC electrical stimulation experiments (40/45), high-impedance microelectrodes (tungsten, 12–15 MΩ; FHC, and A and M Systems) were inserted into the SC through holes 50–75 μm in diameter made in the bone directly overlying the midbrain. To address concerns about current spread, a bipolar electrode (FHC 2 MΩ) was used to deliver current in a subset (5/45) of experiments. The positioning of each electrode was confirmed by listening to neural activity in response to auditory stimulation (see Valentine and Moss 1997). In many penetrations, responses were sampled every 50 μm from 200 μm down to a depth of 400 μm from the dorsal surface of the SC.

The sites of electrode insertion were drawn on a surface map of the SC made for each bat, with the coordinates referenced to a microscope reticule. An electrolytic lesion (1–2 μA, positive current, 3–6 min) was placed at the final stimulation site in each bat, and the tissue was analyzed histologically to confirm the placement of the electrode in the tissue. The topographical organization of evoked responses was assessed in individual bats and for the population. Furthermore, electrical stimulation parameters were varied systematically at multiple sites in the SC in order to determine the relationship between the parameters and the locus of microstimulation, and the characteristics of the elicited behavior.

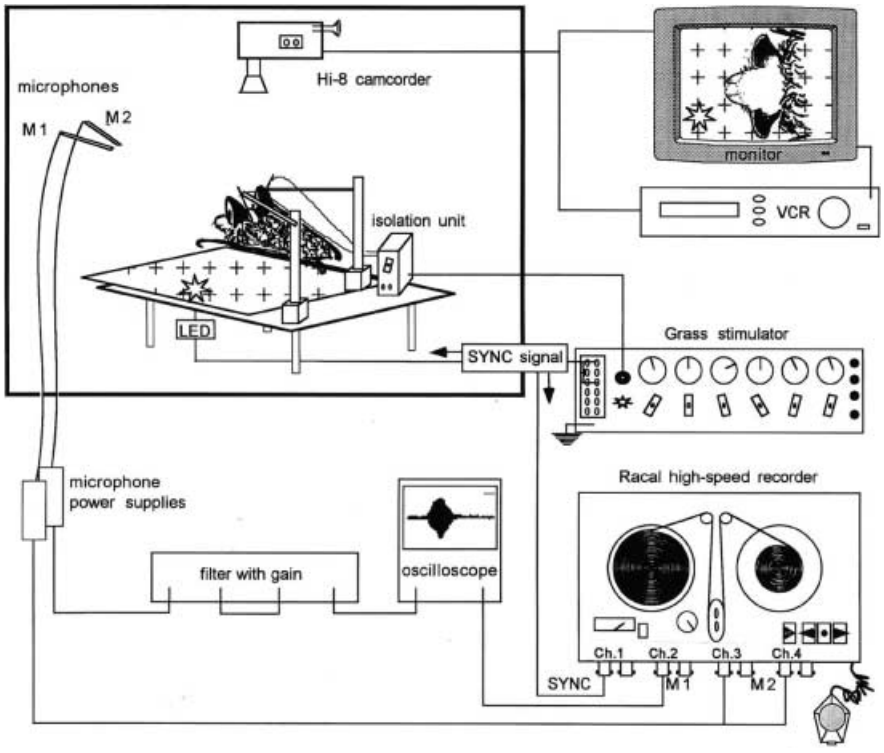


Fig. 1. Experimental set-up for delivering electrical microstimulation to the supercolliculus (SC) and for recording motor responses. Holding device also was used in chemical microstimulation experiments. An additional three-axis manipulator for positioning a Hamilton syringe was used for pressure injections of kainic acid (KA) or saline

Control studies

Electrical stimulation

To determine whether electrically evoked vocalizations in the bat SC differ from those evoked by stimulation of the periaqueductal gray (PAG), a series of control experiments was conducted. A monopolar electrode (impedance approximately 8 MΩ) was advanced through the SC to a depth of 900–1400 μm from the dorsal surface into the PAG. The location of the electrode tip in the PAG was later confirmed histologically. The electrical stimulation parameters were the same as those used to stimulate cells in the SC.

Chemical stimulation

A set of ten chemical-stimulation experiments was also conducted, in which a 500-nl Hamilton syringe needle (part number 7000.5), mounted with a glass micropipette, was advanced by a three-axis micromanipulator (Kopf) to a desired depth in the SC, and an excitatory neurochemical, kainic acid (KA), that activates cell bodies rather than fibers of passage, was delivered by pressure injection (0.5% concentration, 10–20 nl). As part of this study, two bats served as subjects in control experiments, in which physiological saline (10–20 nl) was injected into the SC. Pressure injections of KA or saline were made 300–450 μm from the dorsal surface of the bat SC, with the needle first being advanced 50 μm deeper than the targeted injection site, and then withdrawn by 50 μm to minimize efflux of solution. Injections were carried out at a single site, no more than once in the left and once in the right SC of each subject. The syringe needle was coated with crystals of a lipophilic dye (DiI, Molecular Probes) that aided histological confirmation of the injection site. Micropipette penetrations also were mapped with respect to the surface plane of the SC.

Electrical microstimulation conditions and parameters

In experiments using monopolar electrodes, single and multiple trains of constant current (negative), electrically-isolated twin pulses were generated at a pulse rate of 100–200 Hz by a Grass stimulator (model S48) and stimulus-isolation unit (model PSIU6). Current levels were typically between 4 μA and 15 μA (occasionally up to 40 μA), and the duration of the stimulus-train was varied between 50 ms and 1000 ms at a 2-Hz rate. Data were taken for stimulation pulse durations of 0.3 ms (although durations ranging from 0.1 ms to 0.9 ms were also tested), and the time between the two pulses in the pair was 1 ms. The method used here was similar to the microstimulation technique described to explore vocal-motor responses in the anterior cingulate cortex of the CF-FM mustache bat, *Pteronotus parnellii* (Gooler and O'Neill 1987), and comparable to protocols used by other investigators (Schuller and Radtke-Schuller 1988, 1990; see also Ranck 1975; Asanuma 1979). In the five experiments carried out using bipolar electrodes, the stimulation parameters were as follows: twin-pulse (0.3 ms), 200–400 pulses s^{-1}, 2–15 μA, presented in trains, with a duration of 200 ms at a rate of 2 Hz, negative current. The purpose of these experiments was to determine whether the behavioral responses elicited by bipolar stimulation (with more restricted current spread) were comparable to those elicited by monopolar stimulation, and therefore, the electrical stimulation parameters were not extensively manipulated.

Microstimulation-evoked behaviors were studied under both fixed-head and free-head conditions in a subset of experiments (13 sites using monopolar electrodes). Stimulating current was passed to evoke pinna and vocal responses from several sites before cementing the electrodes in place using cyanoacrylate (Locktite 411) and accelerator (ZipKicker). With the electrodes firmly fixed, the bat's head was released from the restraining mount and stimulating current was passed to elicit a complete set of behaviors, including head movements.

Chemically evoked responses were also studied under both fixed-head (ten sites) and free-head conditions (five sites). In all chemical stimulation experiments, the head was fixed during the pressure injection of KA. The experimenter then removed the syringe needle from the brain and observed the animal's behavior under fixed-head conditions, including the occurrence of any ultrasonic vocalizations. In a subset of five experiments, the influence of head restraint was studied. For this subset, the result of KA injection was first recorded with the head fixed; subsequently, the

head mount was released from the holder, and the bat's behavior again was recorded.

Experimental setup for data acquisition

In the electrical stimulation experiments, the Grass stimulator sent a synchronization signal ("sync" signal in Fig. 1) to both video and audio recording systems. The sync signal triggered an LED visible in the field of the video camera and also was recorded as a brief electrical pulse on a separate channel of a reel-to-reel high-speed audio recorder (Racal Store-4).

The video system used for recording pinna and head movements consisted of a Hi-8 camcorder (Canon H460) mounted above the bat and a television monitor for on-line study of movements. The Hi-8 system recorded video images at 30 frames s^{-1} and using a VHS playback system (Sony DA Pro 4-Head VCR), each video frame was analyzed off-line with a temporal resolution of 33 ms. In two experiments, a digital high-speed video recording system was used (Redlake) that allowed us to record video images of movements at 300 frames s^{-1}. The high-speed video images were stored on VHS tape for off-line, frame-by-frame analysis at a time resolution of 3.3 ms. Individual frames also were scanned as digitized images in grayscale for analysis and manipulation in Adobe Photoshop (version 2.5). Sonar vocalizations were recorded using two microphones (ACO 1/4 inch and QMC Ultra Sound) positioned 10 cm in front of the bat. The ACO microphone was placed centrally and in line with the snout of the immobilized bat. The QMC microphone was oriented about 30° from the center on the side opposite to the stimulation site, in the direction of an elicited, contraversive head movement. The output of each microphone channel was delivered to separate input channels of the Racal tape recorder, and audio signals were recorded at 30 inches s^{-1}. Following KA injections, motor behaviors were noted in the experimenter's log book, and sonar vocalizations were recorded on reel-to-reel tape (Racal Store 4), following the procedures described above.

Analysis of video images and acoustic signals

Off-line analyses were carried out for both video and audio recordings made during microstimulation experiments. Quantitative analyses taken from electrical stimulation experiments included measures of response latency for pinna and head movements and for the production of sonar vocalizations. The time to a stimulated response recorded on video was calculated as the number of frames from the first frame in which the L.E.D. began to illuminate multiplied by the interval between frames (e.g., 33 ms for the 30-Hz camera frame rate). Movement latencies measured from images filmed with the high-speed system were determined directly from a digital image counter, and were ten times the resolution of the Hi-8 system. Vocalization latencies were measured from the onset of the sync signal, marking the time of electrical stimulation recorded on one channel of the audio tape, to the onset of the first emitted sound. In a few cases, the first emitted sound was considerably weaker than the group of sounds that followed. Though an accurate measurement of the frequency structure and pulse duration was not possible for these weak signals, the sound production rate and latency were counted from this first sound.

In the electrical stimulation experiments, we examined how the components of the evoked motor responses depended on both the site and parameters of focal stimulation. The microstimulation parameters – current level, stimulus-train duration, stimulus-train rate, stimulus-pulse rate, and stimulus-pulse duration – were varied systematically to determine how electrical activity influenced the evoked responses. Measures were taken on the following temporal features of the electrically-evoked responses: duration of the motor sequence, the time of occurrence of each component in the sequence and, in the case of head and pinna movements, the latency of the peak excursion of the movement. For the head and pinna movements, descriptive characteristics of the direction and amplitude were noted.

Vocal signals evoked by electrical and chemical stimulation of the bat SC and PAG were played back at 1/32 the recording speed (yielding an effective sampling rate of 604 kHz) for off-line analysis of the sounds. The vocalizations were displayed as time waveforms and as spectrograms, and analyzed using signal processing software (Sona-PC Waldmann: FFT 256 pts, maximum sampling rate of 19.2 kHz). Time waveforms and spectrographic displays of the recorded acoustic signals were used to measure the duration of a sequence of vocalization pulses from the onset of the first to the end of the final sound. The repetition rate of the emitted signals was determined by measuring the interpulse interval from the beginning of one sound to the beginning of the next. The duration of each sound in the sequence, as well as the bandwidth of the fundamental harmonic, was measured at the point where the signal amplitude exceeded the background noise by 12 dB. Calibrated measures of acoustic signal levels were not taken; however, based on a relative scale of dynamic range, the emitted signals were judged to be on the order of 70–100 dB SPL, which is the sound level of emissions produced by *E. fuscus* during echolocation.

Results

General properties of evoked responses

Microstimulation experiments were carried out with monopolar electrodes at a total of 47 sites in 19 adult big brown bats, *E. fuscus* (40 sites in the SC and 7 sites in the PAG). Bipolar electrical stimulation was carried out at an additional 5 sites in the SC of 4 bats. KA also was used to elicit motor activity at 10 sites in 7 bats in the SC. Microstimulation of the SC using monopolar electrical stimulation and chemical stimulation was carried out under conditions in which the head was maintained in a fixed position (fixed-head) or the head was free to move (free-head). Pinna movements and vocalization behavior were monitored during both fixed-head and free-head trials. Table 1 summarizes the responses evoked by each method of stimulation under these conditions.

SC stimulation, delivered electrically or chemically, produced pinna and head movements and sonar cries, while stimulating the PAG evoked vocalizations resembling communication signals and no pinna/head movements. In two chemical stimulation control experiments, physiological saline was pressure-injected, yielding no observable motor responses.

The current threshold for producing a detectable behavioral response in monopolar stimulation experiments was determined using a single train of twin electrical pulses, each lasting 0.3 ms, that were presented at a rate of 100 Hz. The criterion for determining stimulus current threshold in this study was an elicited twitch of the pinna or neck muscle detectable in the video image. Threshold for vocalization was defined as a single vocal signal emitted in response to a single train of the stimulating current. *E. fuscus* did not spontaneously vocalize during testing in the experimental booth, and vocalizations were observed only directly following stimulation. Spontaneous sonar vocalizations were, however, recorded from experimental animals when they were

Table 1. Summary of electrically stimulated and chemically stimulated superior colliculus (SC) sites producing motor responses

	Motor response	Number of sites responding
Monopolar electrical stimulation		
Fixed-head condition ($n = 40$ sites in 14 bats)	Pinna movements	36/40 (90%)
	Pinna movements + vocalizations	16/40 (40%)
	Vocalizations only	0/40 (0%)
	Neck or body movements	19/40 (48%)
	No response	4/40 (10%)
Free-head condition ($n = 13$ sites in 8 bats)	Head movements	11/13 (85%)
	Pinna movements	12/13 (92%)
	Vocalization	10/13 (77%)
	Vocalization in free-head condition only	5/13 (38%)
	No response	0/13 (0%)
	Total vocalizations elicited across fixed-head and free-head conditions	21/40 (53%)
Bipolar electrical stimulation		
Fixed-head condition ($n = 5$ sites in 4 bats)	Pinna movements	3/5 (60%)
	Pinna movements + vocalizations	2/5 (40%)
	Vocalizations only	1/5 (20%)
	Neck or body movements	2/5 (40%)
	No response	2/5 (40%)
Free-head condition		Not tested
	Total vocalizations elicited	3/5 (60%)
Chemical stimulation		
Fixed-head condition ($n = 10$ sites in 7 bats)	Pinna movements	8/10 (80%)
	Pinna movements + vocalizations	4/10 (40%)
	Vocalizations only	0/10 (0%)
	Neck or body movements	7/10 (70%)
	No response	2/10 (20%)
Free-head condition ($n = 5$ sites in 3 bats)	Head movements	4/5 (80%)
	Pinna movements	4/5 (80%)
	Vocalization	4/5 (80%)
	Vocalization in free-head condition only	3/5 (60%)
	No response	1/5 (20%)
	Total vocalizations elicited across fixed-head and free-head conditions	7/10 sites (70%)

allowed to fly in a large room outside the test booth, and these signals were compared with experimentally elicited vocalizations.

Stimulus threshold was lowest for pinna movements and highest for vocalizations; although, as shown below, the difference in the level of electrical stimulation required to produce the two responses was small. The behavioral sequence consisted of pinna movements, elicited with the shortest latency, followed by a head movement and vocalizations, though the complete set of responses was not evoked at every site. While KA studies did not include threshold estimates, the behaviors elicited by application of the excitatory neurochemical were comparable to those elicited by electrical stimulation.

Pinna movements

Pinna responses to microstimulation were characterized by distinct movements of the base of the ear, the thin tip region, and the accordion fold of the auricle. They were reliably elicited under both fixed-head and free-head conditions. That is, the occurrence of a pinna movement did not depend on the mobility of the head. Current thresholds ranged from 5 μA to 26 μA, though typically, the threshold for eliciting movement of the external ear was 8–10 μA. Stimulation at four sites using current levels up to 15 μA did not produce a response. In a couple of experiments, current levels up to 40 μA were used, and a site in the anterior region of the SC required current levels of this magnitude to produce conjugate movements of the two ears and an upward movement of the head. Thus it is possible that a stronger current would have elicited pinna responses at the four sites that produced no response to low levels of stimulation.

Analysis of pinna movements included measures of the latency, direction, and peak amplitude of the movement produced in the contralateral and ipsilateral ears. Movements of the contralateral pinna typically were evoked with a shorter latency and a lower level of stimulation than were movements of the ipsilateral pinna. At threshold, contralateral pinna movements were recorded with latencies on the order of 66–99 ms (2–3 video frames). Above threshold, movements of the contralateral pinna were found to occur within the time of the first frame of the video, suggesting a response latency that was shorter than 33 ms. The results of experiments in two bats using a high-speed digital video system (Redlake, 300 frames s^{-1}; see Materials and

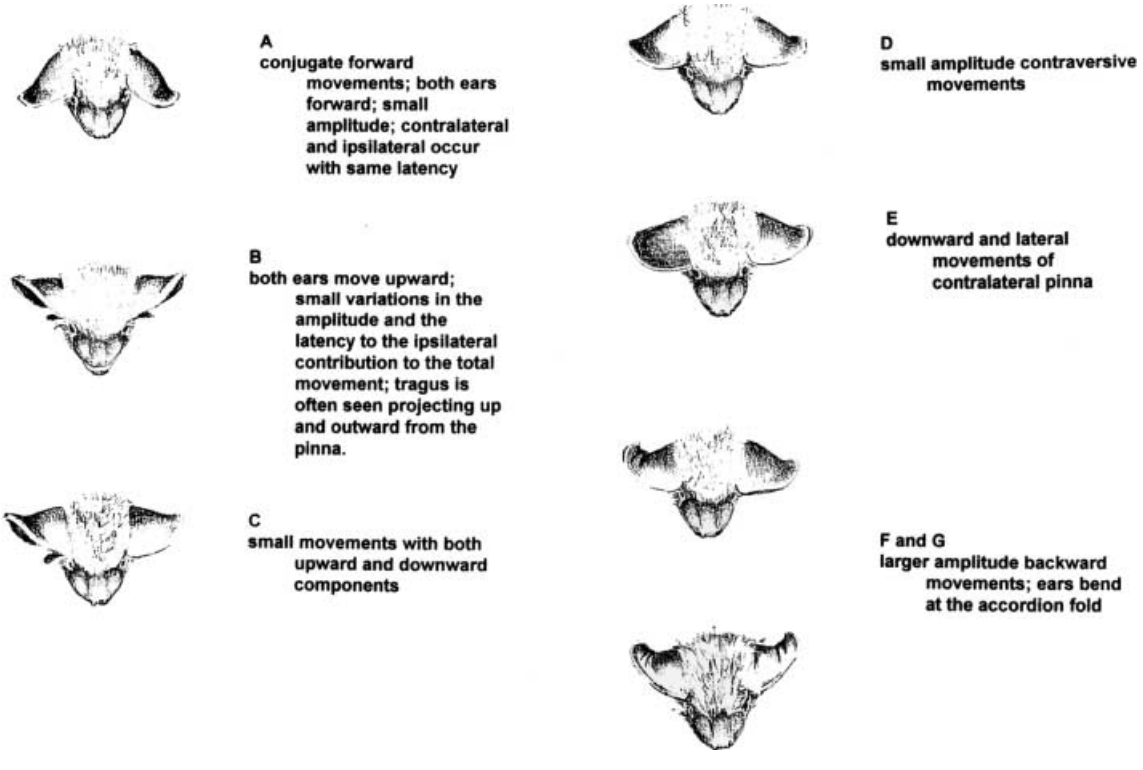
methods) indicate the shortest latency to a twitch of the contralateral ear was on the order of 16–21 ms. For complex patterns of pinna movements evoked from lateral and posterior sites, ipsilateral responses were observed only when the stimulus-train duration was longer than the latency for the ipsilateral component. In these cases, a stimulated movement of the ipsilateral pinna lagged behind the movement produced in the contralateral pinna by as much as 250 ms, and movements of the ipsilateral pinna were not observed when the train duration was shorter than this value. KA injections evoked movements of the pinnae and head, in the direction contralateral to the injection site. Response latencies could not be measured following chemical stimulation.

The direction and laterality of the electrically evoked pinna response depended almost exclusively on the site of stimulation in the SC; it did not vary as a function of changing stimulation parameters, nor with the method of stimulation. At threshold, movements of the pinnae were exclusively contralateral, except at anterior sites that showed conjugate, forward-directed movements of both pinnae, with left and right ear responses occurring at the same time (Fig. 2a). At lateral and posterior sites using suprathreshold levels of current, microstimulation produced movements in the ipsilateral pinna that followed the response in the contralateral pinna, and the amplitude of the ipsilateral movement was typically smaller than that observed for the contralateral ear (Fig. 2f). Moreover, the direction of contralateral and ipsilateral components depended on the locus of stimulation. Relative to the animal's longitudinal body axis, the two pinnae moved in opposite directions at stimulation sites in the anterior and posterior poles of the SC (conjugate, forward movements for anterior sites and non-conjugate, backward movements for posterior sites), or in the same direction at sites between the poles (both rightward for sites in the left SC or both leftward for the right SC, with upward and downward components, depending on the locus of stimulation along the medio-lateral axis; Fig. 2).

While the contribution of the ipsilateral ear and the direction of the movement were specified by the location of the stimulating electrode, the amplitude of the movement vector depended on the site of stimulation, the current strength, and the stimulus-train duration. Figure 3 displays the map of pinna movements on the surface of the SC. Smaller amplitude movements and forward conjugate movements were produced as a result of stimulation in the anterior zone. Stimulation of lateral sites in the bat SC evoked downward movements, and stimulation of medial sites elicited upward movements. In the posterior SC, backward movements were elicited.

The effect of the rate of electrical stimulation on features of the movement also was examined. The amplitude of the pinna movement was greatest at stimulus-pulse rates between 200 Hz and 250 Hz and decreased in size in response to stimulus-pulse rates slower or faster than the optimal rate. The response latency as a function of stimulus-pulse rate followed an inverse relationship, indicating that the strongest behavioral responses occurred at the shortest latencies.

Fig. 2a–g. Characteristics of pinna movements produced by site-specific electrical microstimulation



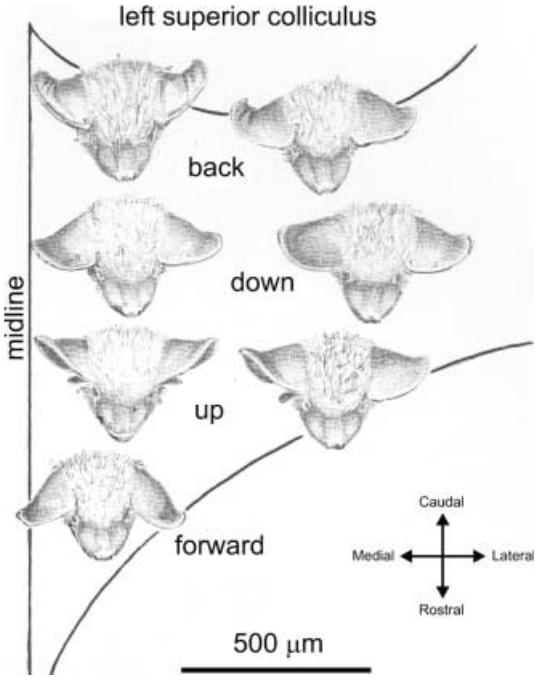


Fig. 3. Composite map of pinna movements on the dorsal surface of the SC

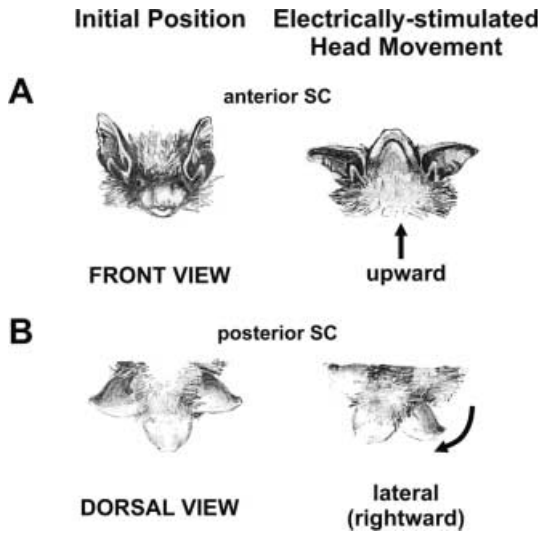


Fig. 4a, b. Sketch of (**a**) an upward head movement and (**b**) a lateral head movement with respect to initial position of the head. Electrical microstimulation (current levels as described in Materials and methods) of anterior sites produced head and pinna movements with upward components. Stimulation of posterior sites in the SC evoked lateral movements of the head and pinna

Electrically and chemically evoked movements under free-head conditions

During free-head trials, each site was tested to determine the role of the SC in producing orienting responses that included head and pinna movements. The minimum current threshold observed for eliciting a head movement was 5 μA, though thresholds up to 10 μA were common, as was the case for pinna movements. Pinna and head movements were typically evoked with the same current threshold, though the pinna response preceded the head component by tens of milliseconds. The shortest latencies for head movements were on the order of 47–53 ms, though longer latencies (between 66 ms and 99 ms as determined with the Hi-8 video system) were common. Thresholds and latencies could not be estimated for the chemically evoked movements in this study.

The directionality of evoked movements followed the trends found for pinna movements under fixed-head conditions. The different movement profiles produced by stimulating electrically at anterior and posterior poles of the SC suggest that the direction and amplitude of head movements are also site-specific. Figure 4a is a drawing of an upward head movement, showing the response observed when the electrode was implanted in the anterior half of the structure. The bottom panel (Fig. 4b) depicts a lateral (rightward) head movement that was produced by stimulating in the posterior half of the left SC. The pinna movements that accompanied the head responses followed the same directional patterns observed under fixed-head conditions. The external ears tipped upward in the first case shown, exposing the tragus to the camera's view (as in Fig. 2b); in the second case, the contralateral pinna moved laterally (as in Fig. 2f).

Electrically and chemically evoked sonar vocalizations

Sonar vocalizations were elicited by electrical and chemical stimulation of the bat SC, and examples are shown in Figs. 5 and 6, respectively. These vocal emissions were produced under fixed-head and free-head conditions, and at 21/40 (53%) of the total number of sites that were tested with monopolar stimulation and 3/5 (60%) of the sites tested with bipolar stimulation. Chemical stimulation elicited vocalizations in 7/10 (70%) of the sites tested.

In Fig. 5a, spectrograms of sonar sounds elicited by monopolar stimulation are presented at three different current levels (10 μA, 12 μA, and 15 μA). The vocalizations produced at every level of current stimulation resembled normal echolocation sounds emitted by *E. fuscus* during the approach phase of insect pursuit (Fig. 5d), though the fundamental was slightly lower in frequency (see Table 2). Bipolar stimulation also elicited sonar vocalizations comparable to the frequency-modulated sonar signals produced by free-flying bats (Fig. 5b), and the characteristics of these vocalizations are indistinguishable from those elicited by monopolar stimulation.

Current thresholds for eliciting vocal responses were 9–10 μA for monopolar stimulation and 13–15 μA for bipolar stimulation. Vocal response latencies under conditions of monopolar-electrode stimulation ranged from 93 ms to 401 ms, and the mean latency for a

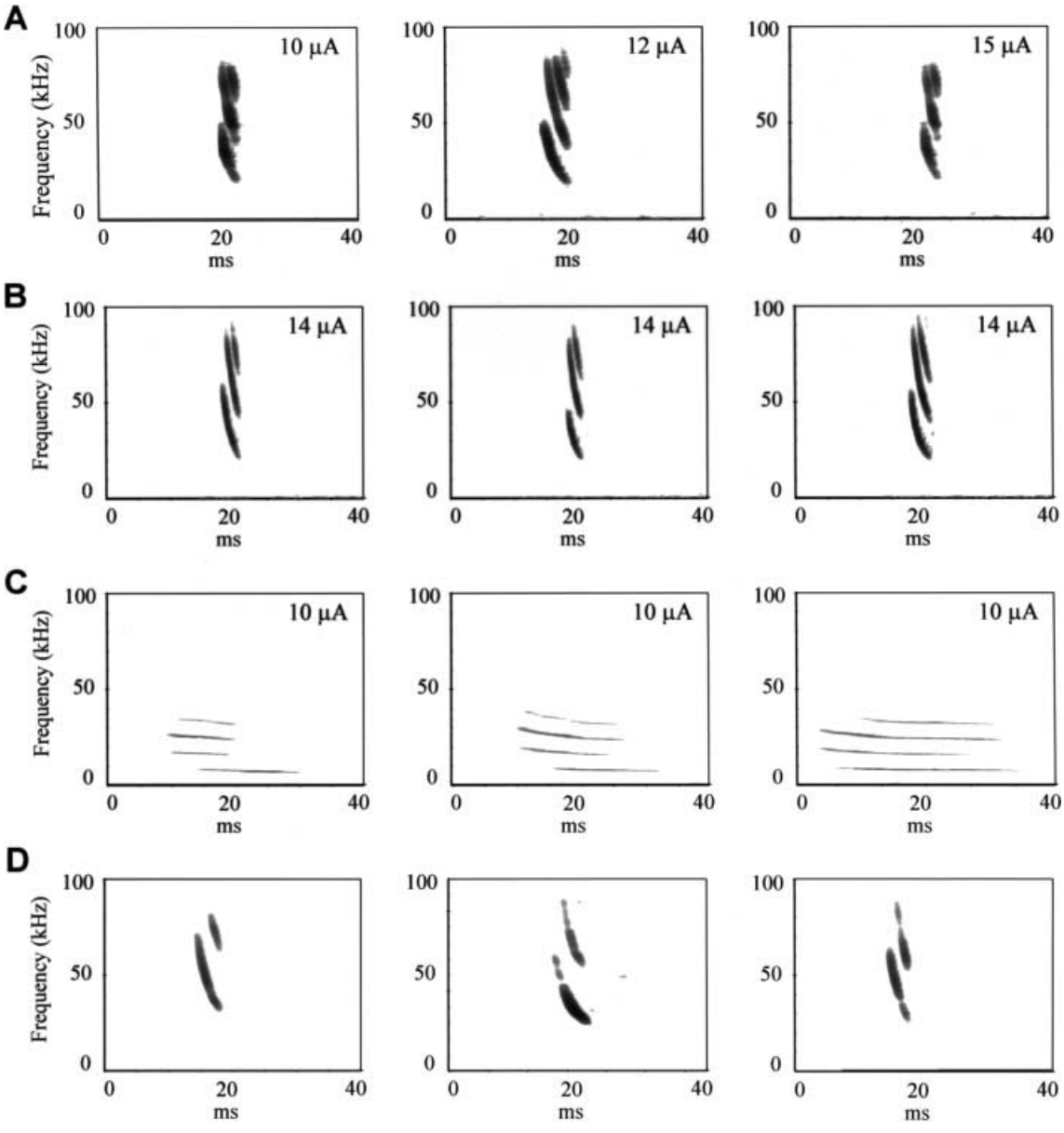


Fig. 5a–d. Representative vocalizations elicited by microstimulation (**a**–**c**) or in a freely flying bat (**d**). Electrical stimulation parameters: single train duration = 400 ms, stimulus-pulse duration = 0.3 ms, stimulus-pulse rate = 200 Hz. **(a)** Monopolar electrical microstimulation. Spectrograms for representative vocalizations elicited at stimulation levels of 10 μA, 12 μA, and 15 μA, (left to right, respectively). **(b)** Bipolar electrical microstimulation elicited vocalizations at three different sites in two different bats. All examples elicited with 14 μA. **(c)** Monopolar electrical microstimulation in the peri-aqueductal greg (PAG) elicited vocalizations resembling those recorded during social interactions among bats. All examples elicited with 10 μA. **(d)** Spontaneously produced vocalizations recorded from a bat, flying freely in a laboratory flight room

response at suprathreshold (12–13 μA) current levels was 195 ± 85 ms. Monopolar stimulation case B-25 showed average response latencies more than two standard deviations above the mean of all monopolar stimulation cases combined, with a mean latency of 323 ± 61 ms. The mean response latency of all data excluding those taken from B-25 was 170 ± 63 ms, which is not statistically different from the response latencies with bipolar stimulation ($t = 0.63$, $P > 0.05$; two-tailed t-test with unequal variances). The mean response latency of vocal responses to bipolar stimulation at 13–15 μA was 162 ± 52 ms.

Pressure injections of KA in the bat SC elicited vigorous vocal behavior from the bat in 7/10 sites tested. Examples of sounds produced by a bat following KA injection are displayed spectrographically in Fig. 6. The duration of each signal is presented in the upper-right corner of the panel. The signal shown in the top panel was recorded 85 s after KA injection, and the signal shown in the bottom panel was recorded 210 s after injection. The vocal signals elicited by chemical stimulation of the bat SC resemble sonar sounds recorded from *E. fuscus* echolocating in the open field (Surlykke

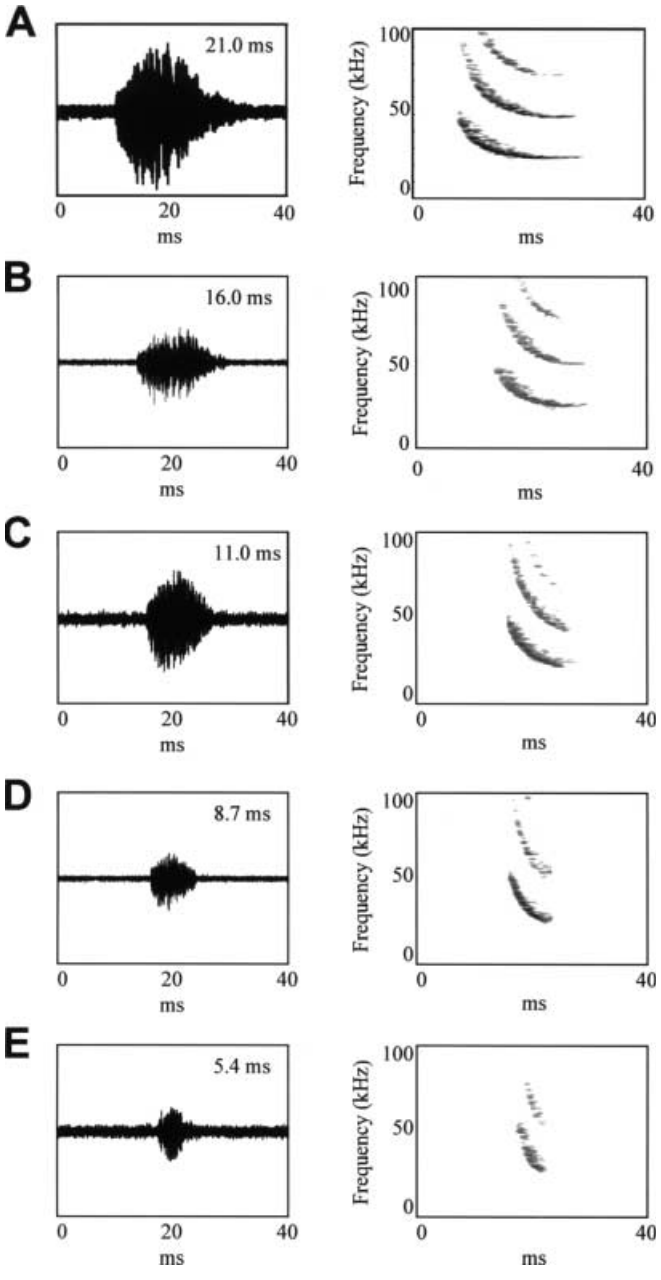


Fig. 6a–e. Spectrograms of representative sonar vocalizations elicited with KA injections in SC. Injections were 10–20 nl of 0.5% KA. (**a–e)** represent sonar emissions of longer to shorter duration elicited by the bat in one bout. The duration of each signal is shown in *upper right corners*. Vocalizations started within 5 s of injection. The longer duration vocalizations were produced early in the recording and progressively decreased in duration until vocalizations stopped. Vocalizations shown were produced between 85 s and 210 s from the time of KA injection

and Moss 2000) or free-flying in the laboratory flight room (Fig. 5d), and resemble the sounds evoked by electrical stimulation (Fig. 5a, b).

When the stimulating electrode was advanced into the PAG (900–1400 μm from the dorsal surface of the brain; see Fig. 10 for histological marking of one stimulation site), vocalizations were elicited at a mean repetition rate of 8.3 Hz, but the spectral and temporal parameters changed dramatically from those produced by electrical and chemical stimulation in the SC. The response latency was also shorter than vocalizations evoked by SC stimulation (mean of 58 ± 3 ms with 10 μA monopolar PAG stimulation). Figure 5c shows examples of sounds elicited by monopolar stimulation of the PAG of this bat species. These signals contain up to five harmonics, and they are lower in frequency (fundamental frequency in the range of 10 kHz), longer in duration (up to 35 ms), and shallower in frequency modulation than the FM sonar signals used by *E. fuscus* for echolocation (where the first harmonic sweeps from about 60–25 kHz in 2 ms during the approach phase of insect pursuit). Vocalizations could be elicited by PAG stimulation at lower current levels (typically 5 μA) and continued beyond the duration of the stimulus train. The PAG-elicited vocalizations thus differ markedly from those elicited by monopolar, bipolar, and chemical stimulation of the SC, and are comparable to communication signals emitted by *E. fuscus* (Moss 1988).

Quantitative analyses of orienting responses and vocalizations produced by electrical and chemical stimulation

The latency to electrically evoked behaviors, including head and pinna movements and vocalizations, decreased as a function of increasing current strength. This relationship between latency and each behavioral component of the evoked response is shown in Fig. 7a. Each curve in this plot follows a similar relationship to increasing levels of electrical stimulation. In this example, a fine measurement of response latency for the pinna and head movements was not available from the 30-Hz video; but by counting the number of frames from the onset of the stimulating current to the first twitch of the neck or ear flap, the latency could be determined within 33.3 ms (1 video frame). Figure 7a also emphasizes the order in which motor responses were executed. Pinna movements typically were initiated first, approximately one frame before the onset of the head movement. Vocalizations occurred with the longest latency, lagging behind the head movement by as little as 33 ms. The tight temporal coupling among these different motor responses and the order in which each behavior is evoked is consistent with the observation that head and pinna movements and sonar vocalizations function together in acoustic orientation behavior.

Quantitative analyses of the vocalizations elicited by monopolar stimulation are presented in Figs. 7, 8, and 9. As the current strength increased above the minimum threshold, the latency to initiate a response decreased; however, response latency began to plateau for suprathreshold current levels (Fig. 7a). Both the latency and the number of vocalizations emitted as a result of stimulation depended on the parameters of stimulation, i.e., train repetition rate (Fig. 7b), train duration

Table 2. Comparison of sensorimotor properties of the SC with insect pursuit behavior

	Functional properties of SC	Approach phase echolocation behavior[a]
Operating range	3-D neurons: 2.30 m ± 1.38 m (13.5 ± 8.1 ms delay), ranging from 0.68 m to 3.40 m (4–20 ms delay)	0.5–3 m from capture
Resolution of image	Coarse delay (range) tuning	Researchers have speculated that after detection, the bat pursues a moving target without fine image resolution until it is at close range
Directional control	Electrical microstimulation evokes orienting movements of the head and pinna; SC may also play a role in orienting flight musculature As the bat flies in pursuit, its head aim and the axis of its directional hearing are maintained on the moving insect target Properties of 3-D neurons: coupling of range axis to directional hearing mechanisms	
Vocal control	Electrical microstimulation elicits vocalization coupled to head and pinna movements	Echo information processing is used to guide vocal production parameters and head/pinna movements
Characteristics of sonar emissions	Pulse duration: 2.7 ± 1.0 ms, ranging from 1.11 ms to 7.00 ms Repetition rate: 15.4 ± 5.5 Hz, ranging from 8.1 Hz to 56.9 Hz Average upper frequency of the first harmonic: 50.0 ± 4.3 kHz; lower frequency: 23.7 ± 4.8 kHz	Pulse duration: typically between 2 ms and 10 ms Repetition rate: typically 10–50 sounds s^{-1} First harmonic in the multiharmonic FM signal sweeps from 60 kHz to 25 kHz

[a]From Webster and Brazier 1965; Kick and Simmons 1984; Surlykke and Moss 2000

(Fig. 7c), and current level (Fig. 7d). At threshold levels of stimulating current one to two echolocation sounds were produced, and at suprathreshold levels, the bat vocalized up to six individual sonar sounds. Increasing the strength or the duration of stimulation generally increased the number of sonar sounds elicited.

Multiple trains of electrical stimulation were used in a few experiments. Typically, three to five trains were administered at rates ranging from 1 Hz to 5 Hz, but trains up to 8 Hz also were tested. At rates exceeding 2 Hz, the bat's vocal behavior was reduced (Fig. 7b). Further, pinna and head movements produced at such high rates showed a peak response to the first train in the sequence followed by smaller amplitude twitches. Response latencies were also longer for faster train rates.

The duration of the stimulus-train produced systematic changes in the features of the behavioral responses. Figure 7c plots the number of vocalizations produced as a function of train duration for two different current levels, 10 μA and 13 μA. The number of sounds elicited as a function of current strength increased regularly (Fig. 7d), but leveled off at higher current levels where spread outside the SC was likely (not shown).

The duration of the vocal-motor sequence was correlated with the duration of the stimulus train in the SC. This relationship was plotted for a subset of stimulation sites, some of which were made in different bats (identified with different symbols, see Fig. 8a). Current levels for these data were 12–13 μA, which was suprathreshold for eliciting a vocal response at each site. While vocal behavior at every SC site where echolocation sounds were elicited showed the same one-to-one relationship for response duration and train duration, the actual number of vocalizations produced during the stimulation period depended on the stimulation parameters (Fig. 8b).

Repetition rates for sonar vocalizations elicited by monopolar SC microstimulation ranged from 8.1 Hz to 56.9 Hz with a mean of 15.4 ± 5.5 Hz. As expected from the plot of stimulation-response duration in Fig. 8a, the repetition rate of sonar vocalizations did not vary consistently with the train duration of stimulating current (Fig. 8c). The sound repetition rate/current strength function showed only a shallow increase, indicating that the repetition rate of sonar emissions approached a maximum only a few microamperes above threshold levels of stimulation (Fig. 8d). The repetition rate of the vocalizations elicited by chemical stimulation of the SC ranged between 4 Hz and 22 Hz, with the higher repetition rates produced when the signal durations were shorter (i.e., as more time elapsed following the KA injection, see for example Fig. 6e).

Other features of the sonar vocalizations that were examined were the frequency and time structure of individual sound pulses. This analysis was carried out for hundreds of sounds and, consistent with earlier experi-

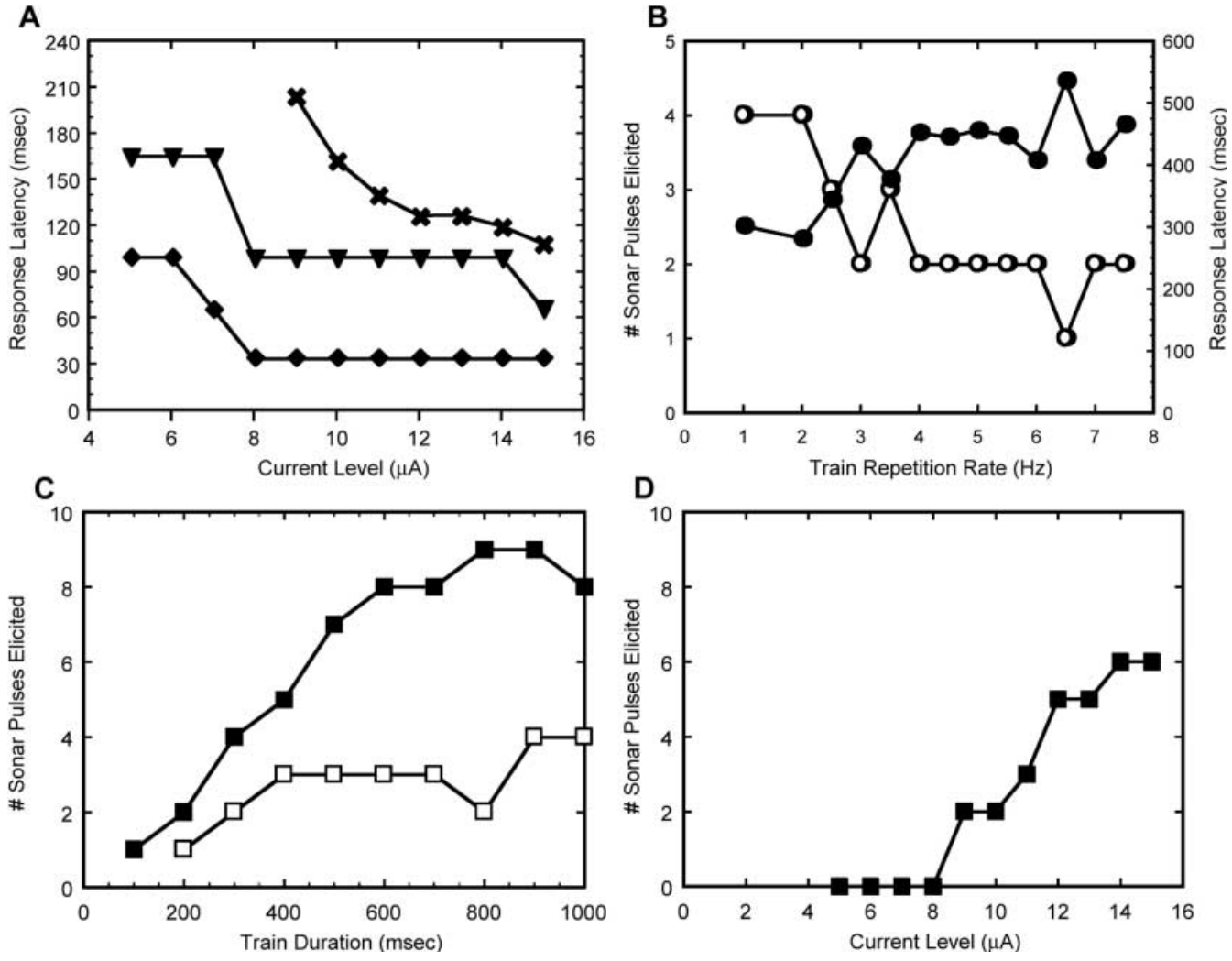


Fig. 7a–d. Stimulus-response profiles from a single bat (case R-63). **(a)** Latency to the initiation of vocalizations (*crosses*), head movements (*filled triangles*) and pinna movements (*filled diamonds*) as a function of current strength. **(b)** The number of vocalizations (*open circles*) and the latency to the first sonar vocalization (*filled circles*) as a function of stimulus-train repetition rate. **(c)** The number of sonar cries as a function of train duration is shown for two different current levels: 10 μA (*open squares*) and 13 μA (*filled squares*). **(d)** The number of sonar vocalizations evoked as a function of current level. The threshold current level for vocalization was 9 μA, for head and pinna movements was 5 μA

ments in the rufous horseshoe bat (Schuller and Radtke-Schuller 1988, 1990), the bandwidth of the sonar emissions evoked by electrical microstimulation changed little as a function of current strength or train duration. The average upper frequency of the fundamental harmonic was 50.0 ± 4.3 kHz, and the lower frequency of the fundamental was 23.7 ± 4.8 kHz (Fig. 9a, b). The bandwidth of echolocation pulses thus was stable over changing levels of stimulating current. The duration of sonar sounds also was mostly stable over changing levels of current strength and train duration, and across different sites of stimulation in the tissue (Fig. 9c, d). The average duration of electrically-evoked sonar cries was 2.7 ± 1.0 ms, ranging from 1.1 ms to 7.0 ms.

Coupling of sonar vocalizations to head movements

Thirteen sites were tested with monopolar stimulation under fixed-head and free-head conditions, eliciting sonar vocalizations at 10/13 (77%). However, at 5 (38%) of these stimulation sites, a vocal response was not evoked under fixed-head conditions, but was produced by the same parameters of stimulation only when the head was free to move. That is, vocal behavior was elicited at low levels of stimulating current (9–10 μA) only when the head was mobile; increasing the current or manipulating other parameters of stimulation under fixed-head conditions had no effect on the production of sonar vocalizations. For example, at a site in the posterior SC, where electrical stimulation evoked vocalizations with a long response latency (mean: 332.0 ± 46.7 ms), the vocal response depended on the occurrence of a head movement. Stimulating at this site under fixed-head conditions evoked pinna movements but vocalizations were not observed for train durations up to 500 ms at 15 μA. When the head was free to move, the same stimulus parameters produced vocalizations at a threshold of 9 μA. At suprathreshold levels of current (13 μA),

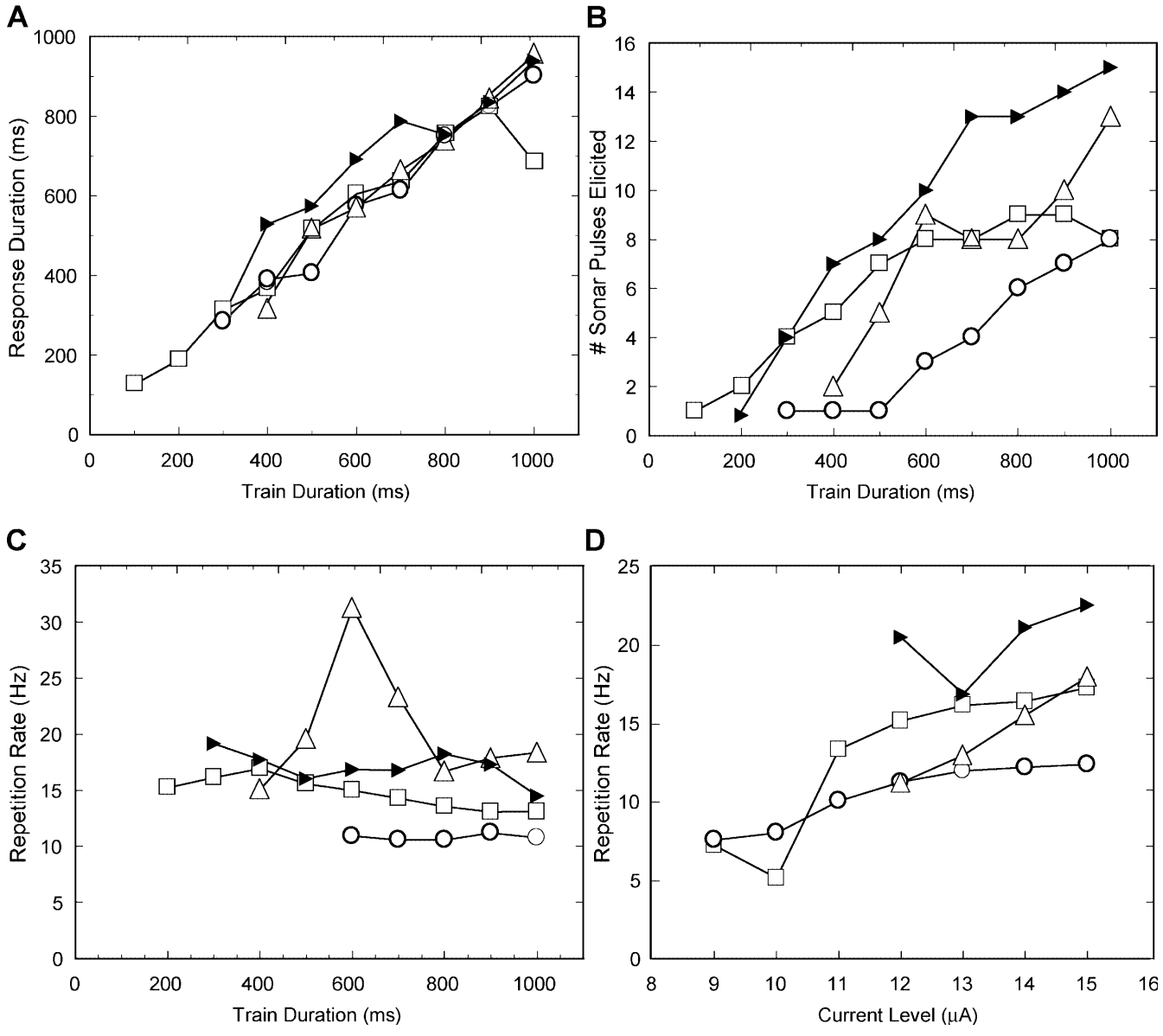


Fig. 8a–d. (**a**) Duration of the vocal-motor sequence as a function of the duration of the stimulus train. (**b**, **c**) Number and rate of vocalizations elicited by microstimulation as a function of train duration. (**d**) Repetition rate as a function of current level. Symbols have following representation: *open circles*: case B-25, *open triangles*: case W-36, *filled triangles*: case P-33, and *open squares*: case R-63. In (**a**–**c**) current level for each case was suprathreshold at 12–13 μA; in (**d**) current was manipulated from threshold to suprathreshold levels (train duration held constant at 400 ms)

vocalizations were produced for train durations of 400 ms or more (Fig. 10).

In five cases, chemical stimulation was tested under fixed- and free-head conditions. At one site tested, KA elicited sonar vocalizations under both conditions. However, in 3/5 (60%) of the sites tested, vocalizations were elicited by KA application only after the animal was released from the head holder and allowed to move freely. In these cases, the animal turned its head and body in the direction contralateral to the injection site while making rapid movements of its external ears. Vocalizations were emitted, showing characteristics of sonar signals emitted from free-flying *E. fuscus* (Surlykke and Moss 2000). All of the behaviors elicited by KA stimulation of the bat SC resembled those elicited by electrical stimulation, but the behaviors occurred continuously over several minutes. Neither pinna movements, head movements, nor vocalizations were elicited in control experiments with saline injections in the bat SC.

Topography of electrically evoked responses

The locations of sites tested for the production of orienting motor responses elicited by low current monopolar stimulation in the SC are shown in Fig. 11. Pinna movements were evoked by microstimulation at 36/40 sites throughout the extent of the SC, and a map of

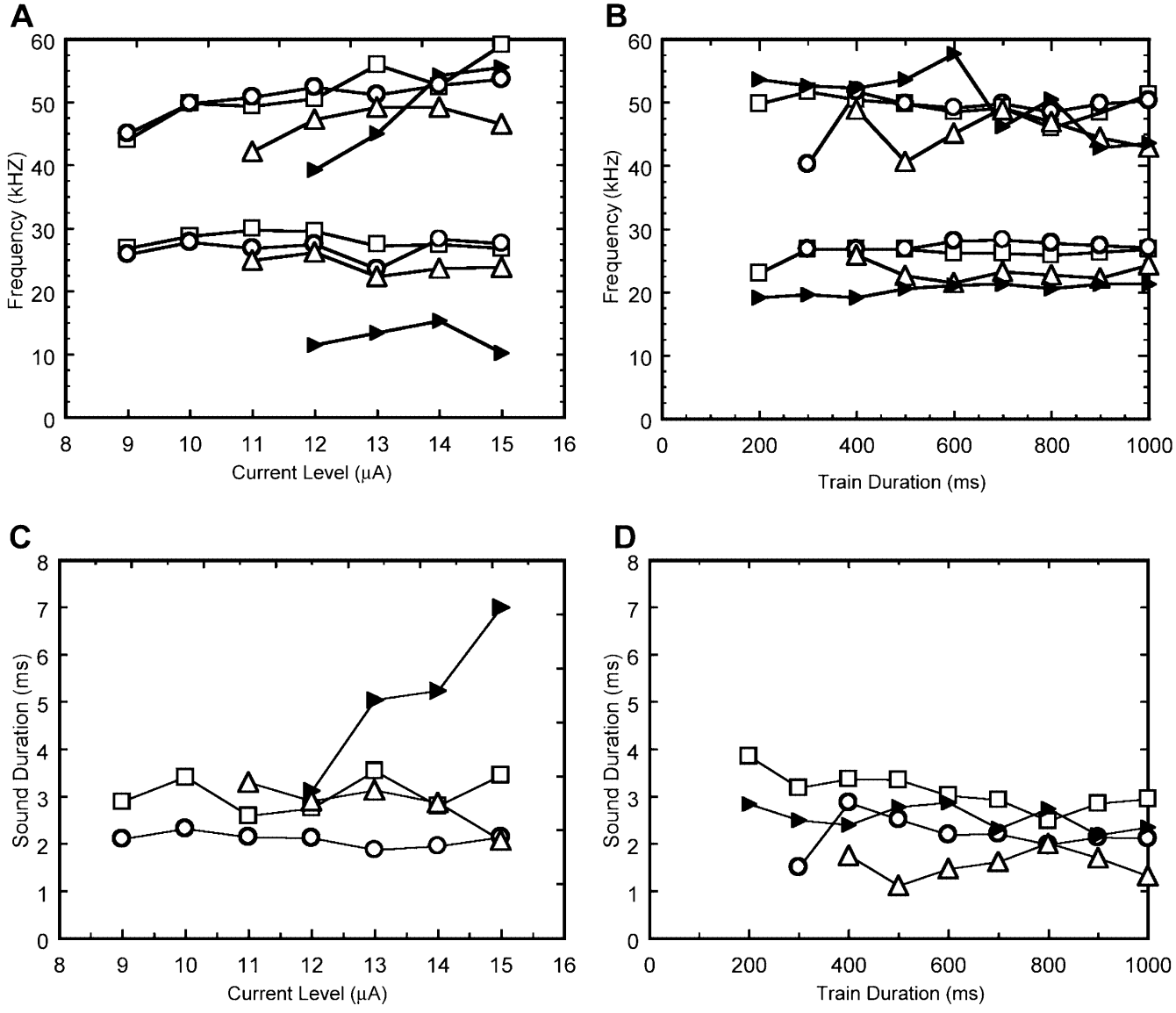


Fig. 9a–d. Average start and end frequencies for fundamental of sonar emissions evoked by microstimulation as a function of current level (**a**) and stimulus-train duration (**b**). (**c**, **d**) Average duration of individual sonar pulses evoked by microstimulation as a function of current level (**c**) and stimulus-train duration (**d**). Each curve represents data taken at different sites in the SC, and symbols have the following representation: *open circles*: case B-25, *open triangles*: case W-36, *filled triangles*: case P-33, and *open squares*: case R-63. In plots (**a**) and (**c**) current levels were manipulated from threshold to suprathreshold values. In plots (**b**) and (**d**) current levels were suprathreshold (10–13 μm)

pinna movements was found (see Fig. 3). Sites in which the electrically evoked responses included head and pinna movement components were found throughout the structure, and the metrics of the head movement responses corresponded to the location of the stimulating electrode. The maps of pinna and head movements were consistent with the functional organization of the SC in other species: upward and forward movements were found medially and rostrally; downward and backward-lateral movements mapped to lateral and caudal sites. Sites that produced vocal output were not clustered nor found to map according to any parameter of the vocal response.

Discussion

Motor responses evoked by microstimulation of the SC

The characteristics and topography of the evoked motor responses in the SC of the echolocating bat are consistent with the motor organization of eye, head, and pinna movements found in other species (e.g., Stein and Clamann 1981; Harris 1980; Robinson 1972). In addition to head and pinna movements, SC microstimulation elicited vocalizations, a result not observed in other vertebrates but previously demonstrated in the rufous horseshoe bat, *R. rouxi* (Schuller and Radtke-Schuller 1988, 1990). The electrically elicited SC vocalizations in our study were frequency-modulated, multiharmonic,

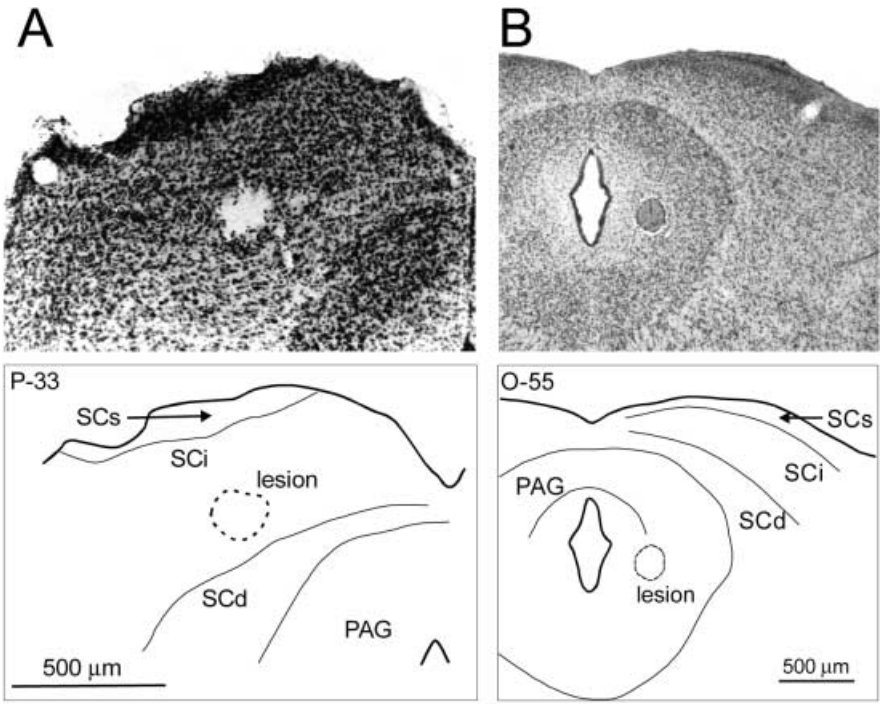


Fig. 10a, b. **(a)** Lesion marking the site of microstimulation in the intermediate left SC of bat P-33. Stimulation at this site produced pinna movements under fixed-head and free-head conditions, lateral head movements, and vocalizations in the free-head, but not the fixed-head, condition. **(b)** Lesion marking the site of microstimulation in the left PAG of bat O-55. Stimulation at this site evoked vocalizations that were longer in duration, lower in frequency and contained more harmonics than the sounds elicited by SC stimulation (see Fig. 5c). Note that the magnification is different in panels **(a)** and (b)

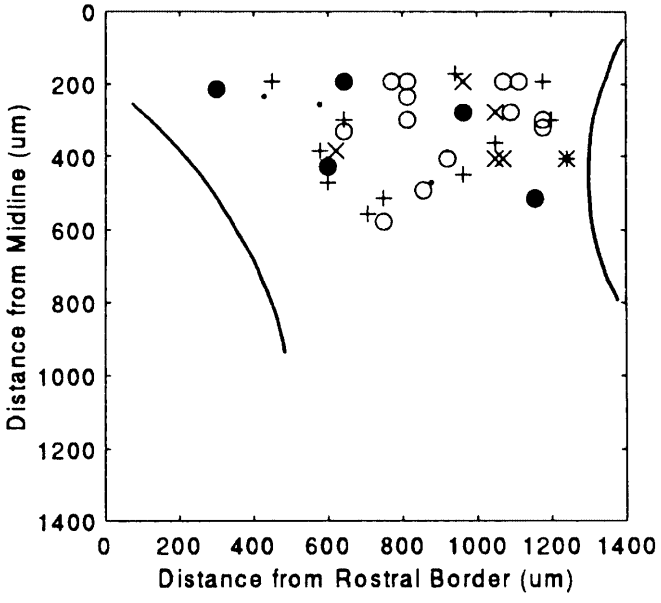


Fig. 11. Location of sites tested with monopolar stimulation for the production of orienting motor responses in the SC. Different symbols correspond to the motor responses evoked at each site. Pinna movements (*open circles*), head and pinna movements (*asterisks*), pinna movements and vocalizations (*plus signs*), head/pinna movements and vocalizations (*crosses*), head/pinna movements and vocalizations only under free-head conditions (*filled circles*), no response (*dots*)

and short duration (on average < 3 ms) echolocation sounds, emitted at a mean rate of about 15 Hz. Additionally, the evoked sound emissions were sometimes coupled to a fixed-direction movement of the head and always co-occurred with movements of the external ears.

The properties of electrically stimulated pinna and head movements depended on both the location of the electrode tip and the parameters of the stimulating current, a finding that is consistent with results of microstimulation experiments in other species (e.g., Stein and Meredith 1993; Stanford et al. 1996). The directionality, magnitude, and laterality of the evoked motor responses were linked to the site of SC stimulation, revealing a topographic organization with respect to pinna and head movements.

At suprathreshold current levels, properties of the elicited behavior, such as response latency and amplitude (for vocalizations, amplitude corresponds to the number of sounds in the evoked sequence), showed a systematic relationship to the parameters of electrical stimulation. With increasing levels of applied current stimulation, motor responses of greater amplitude and shorter latency result from an increased recruitment of active neuron pools.

Estimates taken from Ranck (1975) suggest that current spread for monopolar stimulation of 15 µA or less in these experiments would be no more than 400 µm from the electrode tip. An obvious concern is the possibility that the vocalizations elicited by microstimulation in the SC resulted from current spread to the adjacent PAG and not from activation of neurons in the SC. Several lines of evidence suggest that the vocalizations elicited by microstimulation in the SC come from direct activation of SC neurons. First, bipolar stimulation (with more restricted current spread than monopolar stimulation) at a depth of 300–400 µm from the dorsal surface of the SC and up to 500 µm from the border to the PAG also elicited the production of sonar vocalizations (Fig. 5). Second, pressure

injections of 10–20 nl of 0.5% KA also elicited sonar vocalizations (Fig. 6), while control injections of saline failed to elicit vocalizations of any kind. Chemical stimulation with KA serves to activate cell bodies in the vicinity of the injection site and not fibers of passage, eliminating the possibility of activating fiber tracts that project to structures outside of the SC. Finally, direct electrical stimulation of the PAG did evoke vocalizations, as observed in other species (e.g., Jürgens and Pratt 1979), but the elicited vocalizations had very different spectral and temporal features than those elicited by SC stimulation (Fig. 5c). The vocalizations elicited by stimulation of the PAG could be classified as communication calls (see Moss 1988), with durations up to 35 ms, multiple harmonics, shallow frequency modulation, and fundamental frequencies in the range of 10 kHz, well below that of the FM sonar signals used by *E. fuscus* for echolocation (first harmonic sweeping from about 60–25 kHz in 2 ms during the approach phase of insect pursuit).

Interestingly, the production of vocalizations depended on the mobility of the head in a subset of experiments in which behaviors elicited by SC stimulation were studied under fixed- and free-head conditions. In 5/13 (38%) of the cases where vocalizations were evoked by monopolar stimulation and in 3/5 (60%) of the cases with chemical stimulation, such co-dependency was observed. At the electrical stimulation sites, the properties of elicited pinna movements were identical under fixed-head and free-head conditions using the same stimulating parameters, assuring us that stimulation was taking place at the same locus. Using chemical stimulation, pinna movements elicited under free-head conditions were also indistinguishable from those elicited under fixed-head conditions. Under free-head conditions, chemical and electrical stimulation evoked head turning in the direction contralateral to the injection site. Evoked vocalizations, when also produced in the free-head condition, resembled those elicited in the fixed-head condition.

Further evidence that electrical stimulation of the SC activates neurons for the production of specific behaviors comes from considering quantitative aspects of the response. The results reported here are consistent with the estimated chronaxies, or membrane time constants, of central nervous system neurons in a host of mammals (Ranck 1975). In the bat anterior cingulate cortex where microstimulation yielded vocalizations, the mean chronaxie was measured in current strength-duration curves as 0.358 ms (Gooler 1987), which is close to the value estimated in the present study. Indeed, the electrical-pulse duration and motor response curves show non-monotonic functions, with 0.3 ms stimulus duration producing the peak response. Finally, the relationships between stimulation conditions and motor responses follow those described for current strength and the metrics and kinematics of head movements evoked from the tectum of the barn owl (DuLac and Knudsen 1990). That is, the properties of the stimulated motor response approach a plateau value with an increase in current strength or a change in some other electrical-pulse parameter.

Movements of contralateral pinnae were elicited with the shortest latencies. Based on high-speed digitized video images, these were judged to be on the order of 16–21 ms. At sites where the response in the contralateral pinna was dominant, a stimulated movement of the ipsilateral pinna lagged by as much as 250 ms, suggesting the ipsilateral movement may be mediated by activity that spread to the opposite SC via the commissural pathway. At anterior sites where forward, conjugate movements of the pinnae were produced, the difference in response latencies of contralateral and ipsilateral pinnae was negligible. Though pinna movements elicited by microstimulation of the SC in bat *R. rouxi* (Schuller and Radtke-Schuller 1988, 1990) and in the cat (Stein and Clamann 1981) have been observed, latency measurements were not reported.

For head movements, the shortest latencies were on the order of 47–53 ms, though longer latencies (between 66–99 ms as determined with the Hi-8 video system) were more common. The latencies to an evoked head movement in the bat are comparable to values reported for the cat (Harris 1980; Roucoux et al. 1980) and primates (Cowie and Robinson 1994).

Vocalizations were elicited following the initiation of responses of the pinna and head. The shortest vocalization latencies found were on the order of 93–126 ms, though the time lag between the onset of an evoked head movement and the first sonar cry in a vocal sequence was as little as 33 ms or one video frame. In the rufous horseshoe bat, *R. rouxi,* microstimulation of the SC produced vocalizations with latencies ranging from 30 ms to 80 ms (Schuller and Radtke-Schuller 1988, 1990). The difference in evoked vocalization latency for the two bat species may be related to differences in their vocal production systems. In contrast to the brief, FM sounds emitted by *E. fuscus*, the horseshoe bat uses a long, CF sonar signal in combination with a brief, comparatively shallow FM component. The CF component carries velocity or movement information as Doppler-shifts in the returning echoes (e.g., Goldman and Henson 1977; Schnitzler et al. 1983; von der Emde and Menne 1989; Kober and Schnitzler 1990). While both species of bat possess specialized laryngeal mechanisms for vocal production, tension in the cricothyroid muscle, which is related to the control of frequency in the transmitted echolocation signals, is regulated differently (Schuller and Rübsamen 1981; Suthers and Fattu 1982). In CF-FM bats, activity in the superior laryngeal nerve (SLN) innervating the cricothyroid begins 30–50 ms prior to the emission and is maintained throughout the long, CF component of the signal (Schuller and Rübsamen 1981). In the FM-bat, SLN activity begins about 50 ms prior to vocalization and ends before the vocalization starts, thus creating a pattern of rising and falling tension in the cricothyroid muscle that is correlated with the broadband FM sweep

(Suthers and Fattu 1973, 1982). In addition, the number of SLN fibers innervating the cricothyroid muscle is greater for CF-FM bats than it is for FM-bats (Kobler 1983). These two differences may help explain the longer latencies observed here.

The latency range reported by Schuller and Radtke-Schuller (1988, 1990) overlaps with latencies reported for sonar vocalizations elicited from the midbrain reticular formation (MRF) in the mustached bat, *Pteronotus parnellii*, and the myotis bat, *Myotis austroriparius* (Suga et al. 1973; Suga and Yajima 1988), and from the PAG of *Pteronotus* (Suga et al. 1973; Suga and Yajima 1988) and *R. ferrumequinum* (Rübsamen and Schuller 1981). In the horseshoe bat, the SC projects reciprocally to the PAG and to the paralemniscal tegmental area (Metzner 1996) and indirectly to motor nuclei that control pinna movements (the facial nucleus), head movements (neck motoneurons in the spinal cord), and vocalizations (nucleus ambiguus) (Henkel and Edwards 1978; Kobler 1983; Huerta and Harting 1984; Metzner 1996). In *E. fuscus*, SC efferents project to the paralemniscal area (PLa) and the cuneiform nucleus (CUN); reciprocal projections exist with contralateral SC, substantia nigra (SN), pars reticulata and pars compacta, inferior colliculus (IC), zona incerta, and PAG. The SC of *E. fuscus* also receives projections from the auditory cortex and the olivary and periolivary complex (Sinha et al. 2000). Based on what is known about this circuitry, the SC signal for the control of acoustic orientation by sonar would be delivered to pre-motor and motor centers, and the latencies reported in the present study are consistent with a pre-motor, integrative function for the bat's SC.

Orienting gaze: a comparative, neuroethological approach

The convergence of pinna and head movements with the production of sonar vocalizations helps to bridge the gap between our understanding of the role of the SC in gaze control and in echolocation, a specialized acoustic orienting system. The process of adapting vocal output and tracking behavior in response to auditory input during echolocation is in many ways parallel to the motor control of eye movements in response to sensory input, with eye movements being the most well-understood response of a set of related orienting behaviors that also includes head and pinna movements. Humans, monkeys, and other animals correct for the difference between the current position of the eye and the perceived spatial location of an object by making a quick eye movement called a saccade (reviewed in Sparks 1986). Saccadic eye movements allow an animal to look to an object and to maintain its image on the retina in the region of highest visual acuity. In echolocating bats, it has been suggested that the SC plays a major role in auditory rather than visual (saccadic eye movement) orientation (Covey et al. 1987; Valentine and Moss 1997). The bat uses acoustic information to navigate through space and pursue and capture insect prey in darkness. The presence of a novel auditory stimulus, in the form of a reflected echo, directs head and pinna movements toward the stimulus source; in addition, the bat changes the features of its sonar vocalizations that facilitate reception of additional acoustic information.

The analogous relationship between sonar orientation in echolocating bats and orienting gaze in other species is supported by comparing the bat's coordinated process of vocal production and directional hearing to behaviors observed in a host of other species. For example, microstimulation has been applied to the SC of rodents and the optic tectum of anurans, yielding results that support a broad definition for what constitutes an adaptive gaze shift. In the rat, orienting and withdrawal responses were elicited by electrical stimulation (Sahibzada et al. 1986) and by microinjection of glutamate into the SC (Dean et al. 1988a, 1988b). In toads, stimulating the different regions of the tectum produces one or more of six behaviors typical of the prey-capture sequence, including orientation, approach, visual fixation, snapping, swallowing, and oral grooming (Ewert 1970, 1984). In species that use infrared cues or electrolocation to sense, navigate and hunt, similar correspondences between sensory modality and species-appropriate motor responses have been noted (for pit viper, see Newman and Hartline 1981; for electric fish, see Bastian 1982). Like the echolocating bat, which tailors the features of its vocal emissions in response to acoustic input, electrolocating animals modulate the frequency of their electric organ discharges to avoid jamming by neighboring conspecifics (Heiligenberg 1991). In other words, electric fish regulate their electromotor responses to facilitate processing of electrosensory inputs, and the tectum is thought to play a role in this response.

A model of SC function in the echolocating bat

The idea that the SC of the echolocating bat plays a role in acoustic orientation by sonar is supported by a two-pronged approach that considers not only the motor output that can be evoked, but also the sensory activity that can potentially drive this motor output. Using extracellular recording methods and auditory stimulation under free-field conditions, SC neurons were studied for their selectivity to the spatial location of an acoustic target (Valentine and Moss 1997). Two-dimensional auditory neurons responded selectively to the azimuth and elevation of a sonar sound source, while a second class of cells (3-D neurons) responded selectively to target azimuth, elevation, and range (the latter encoded as a time delay between a simulated outgoing sound and its returning echo). In the bat, changes in echolocation behavior are closely tied to target distance; thus a feedback process that integrates sensory and motor signals driving acoustic orientation by sonar must include information about a target's azimuth, elevation, and distance.

Here, we present a model of the role of the SC in acoustic orientation by sonar that is based on the response properties of SC neurons and the characteristics of electrically-evoked behaviors. The bat orients toward targets using mechanisms common to other species – mechanisms derived primarily from the well-studied saccadic eye movement system. We suggest that SC neurons that are selective to target locations in azimuth and elevation may function to direct the aim of the head and pinnae to a desired position. As diagrammed in Fig. 12a, a signal of motor error, derived from the current position of the head and pinnae and from information about the location of a target (desired position of the head and pinnae), may be processed by SC circuitry and relayed to premotor and motor centers that encode the appropriate commands to the muscles. A "moving hill" of neuronal activity across the SC is thought to form the mechanism by which a population response is translated into discrete and specific movements of the sensory organs (Munoz et al. 1991; Guitton 1992; Port et al. 2000; see also Sparks 1993).

The bat's biological sonar system provides it with additional acoustic cues for localizing objects in space along the distance (echo delay) axis, as well as in azimuth and elevation. SC neurons tuned to three-dimensions of space, possibly interlaid as discrete modules within a two-dimensional spatio-sensory architecture, may interact to direct motor output (vocal and orientation responses) appropriate for both the bat's position and distance relative to the target. The activity of SC neurons may carry information about a sonar target's spatial coordinates in three-dimensions, shown in Fig. 12b, as a facilitated population response. As echoes return from different target distances, the temporal and spatial pattern of neural discharges in the SC may encode the dynamic relationship between the bat's current position with an estimate of its desired position relative to the source of the target. This dynamic motor error signal could be used to guide appropriate head and pinna movements and vocalizations appropriate for tracking an object.

Relationship of SC function to insect pursuit behavior

The pursuit of insect prey is a relatively stereotyped sequence of behaviors in many species of bats (Griffin 1958). Simmons and Kick (1984) describe different phases to the pursuit maneuver of FM-bats: detection, approach-tracking, and insect interception (terminal). Each phase represents a different perceptuo-motor challenge to the bat, and the output of its vocal production, audiomotor, orientation, and flight systems adapts to the requirements of the different phases of insect pursuit and capture. Based on the results of our extracellular recording and microstimulation data, we postulate a role for the SC in sensorimotor coordination that operates during the approach phase of insect pursuit (Table 2; see also Valentine and Moss 1998).

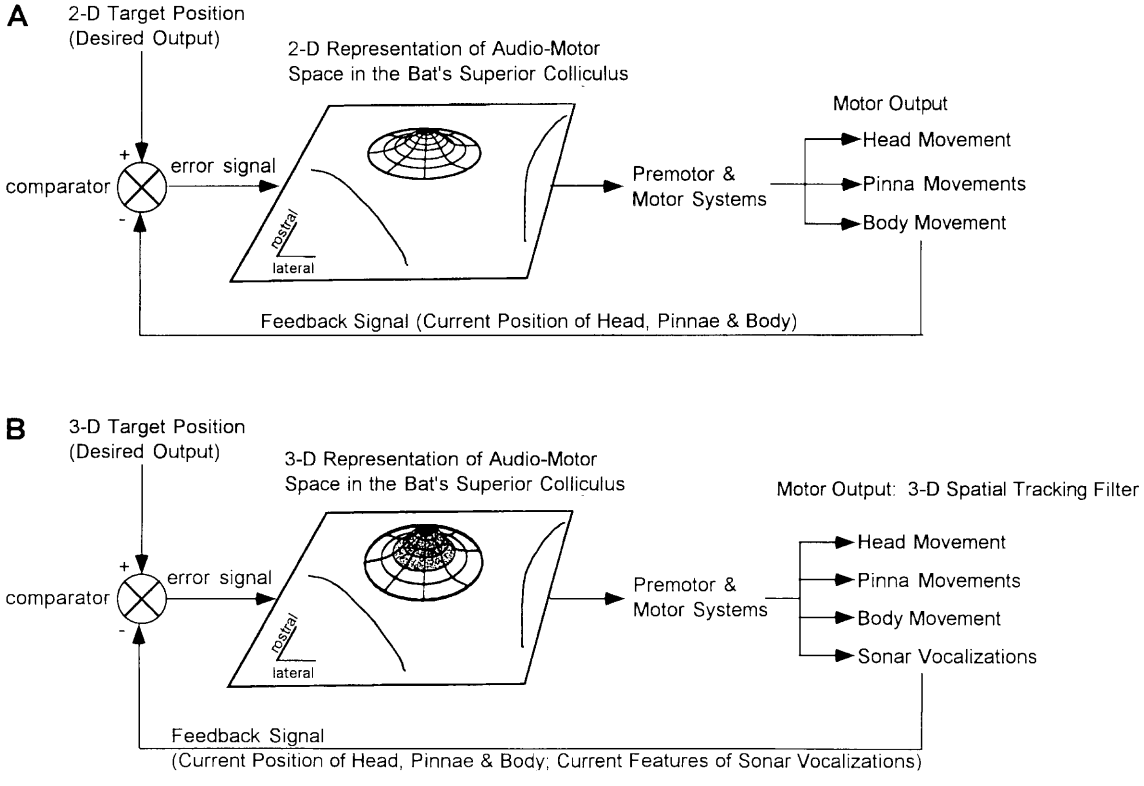


Fig. 12a, b. (**a**) A model diagramming the role of the SC in the translation of two-dimensional, and three-dimensional (**b**) spatial-acoustic information into signals that produce adaptive changes in acoustic gaze

During the approach phase, in which the bat is between 0.5 m and 3 m from the target, the bat transmits multiharmonic FM sounds at a rate of 10–50 sounds s^{-1} that are each 2–10 ms in duration, sweeping from approximately 60–25 kHz in the first harmonic (Surlykke and Moss 2000). Over closing distance and in response to information contained in the returning echoes, the bat modifies the characteristics of its sonar emissions, and these adaptive changes improve ranging accuracy and facilitate prey capture.

That the SC plays an important role in approach-tracking behavior is supported both by the response properties of SC neurons (Valentine and Moss 1997) and by the results of the microstimulation experiments reported here. In particular, the representation of delay sensitivities in SC neurons corresponds to the operating range of this behavioral phase. Furthermore, the characteristics of the elicited vocalizations, coupled to translations of the head and pinnae, reflect the features of emissions produced by the bat at 0.5–3 m from the closing target. Given that an echolocating bat can actively control the spatial information that drives its behavior, both by directing its hearing and by modulating the acoustic structure of its outgoing sonar emissions, we propose that these functionally-related sensorimotor feedback systems share a common neural interface that includes the SC in a larger audiomotor control circuit.

The sensorimotor function of the bat SC follows a general mammalian plan, while it also supports specializations that may be important for acoustic orientation by sonar. The sensorimotor specializations reported in the current study may play an important role in coordinating acoustic information about the position of a target with premotor circuitry that permits a bat to adjust the position of its head and pinna and the features of its sonar vocalizations in response to spatial information contained in sonar echoes. These findings from a specialized animal system contribute to our understanding of the general principles guiding the functional organization of the vertebrate midbrain.

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