

Midbrain auditory selectivity to natural sounds

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This study investigated auditory stimulus selectivity in the midbrain superior colliculus (SC) of the echolocating bat, an animal that relies on hearing to guide its orienting behaviors. Multichannel, single-unit recordings were taken across laminae of the midbrain SC of the awake, passively listening big brown bat, *Eptesicus fuscus*. Species-specific frequency-modulated (FM) echolocation sound sequences with dynamic spectrotemporal features served as acoustic stimuli along with artificial sound sequences matched in bandwidth, amplitude, and duration but differing in spectrotemporal structure. Neurons in dorsal sensory regions of the bat SC responded selectively to elements within the FM sound sequences, whereas neurons in ventral sensorimotor regions showed broad response profiles to natural and artificial stimuli. Moreover, a generalized linear model (GLM) constructed on responses in the dorsal SC to artificial linear FM stimuli failed to predict responses to natural sounds and vice versa, but the GLM produced accurate response predictions in ventral SC neurons. This result suggests that auditory selectivity in the dorsal extent of the bat SC arises through nonlinear mechanisms, which extract species-specific sensory information. Importantly, auditory selectivity appeared only in responses to stimuli containing the natural statistics of acoustic signals used by the bat for spatial orientation—sonar vocalizations—offering support for the hypothesis that sensory selectivity enables rapid species-specific orienting behaviors. The results of this study are the first, to our knowledge, to show auditory spectrotemporal selectivity to natural stimuli in SC neurons and serve to inform a more general understanding of mechanisms guiding sensory selectivity for natural, goal-directed orienting behaviors.

stimulus selection | superior colliculus | auditory grasp | neuroethology

Goal-directed orientation depends on selective sensory processing to guide appropriate movements, such as saccades, head turns, or reaching to grasp a target (1). Previous work suggests that the midbrain superior colliculus [SC; SC of mammals or optic tectum (OT) of other vertebrates] plays a central role in integrating multimodal sensory information for planning and executing goal-directed orienting behaviors (2). Although there is strong evidence that the SC encodes stimulus location to guide orienting movements (3), other work suggests that the SC also mediates target selection and attention: SC recordings reveal shared neural control over attended locations in space and premotor commands to shift gaze to these locations (4–6). Moreover, neurons in the OT of the barn owl respond categorically to multimodal sensory stimuli as “salient” and “other” (7, 8), a categorization that assists in target selection. These findings implicate the SC in both stimulus selection and orientation. Our understanding of the role of the SC in the selection of natural stimuli is, however, incomplete, and to bridge this gap, we investigated auditory selectivity in the SC of the echolocating bat, an animal that shows robust stimulus selection to guide its adaptive orienting behaviors (9).

Auditory selection and attention to sonar targets have been inferred at the behavioral level in echolocating bats from their adaptive control of the signals used to probe the environment (9). However, neural mechanisms of auditory stimulus selection in bats have not been investigated. Earlier neurophysiological studies of auditory processing in the bat SC used simplified acoustic stimuli that only approximated natural echolocation signals (10, 11). Although past research revealed important properties of bat SC neurons, recent studies in other animals

suggest that responses to simplified acoustic stimuli may not reliably predict responses to natural sounds (12, 13). Additionally, in the bat auditory cortex, the temporal dynamics of sounds have been shown to affect auditory response profiles (14, 15), further substantiating the importance of biologically inspired stimuli to assess and understand brain activity in light of its evolved function. It is for this reason that the bat serves as a valuable model for understanding the dynamics of stimulus selection in the SC. The sensory stimuli presented to the bat can recreate natural listening experiences under very controlled experimentation. By manipulating biologically relevant signals with fine control, basic principles of natural stimulus selection in the SC can be uncovered.

Here, we report on auditory responses of SC neurons in the echolocating big brown bat, *Eptesicus fuscus*, to natural and artificial acoustic stimuli, with the goal of understanding the processes that contribute to auditory stimulus selection. Considering the importance of auditory computation to the bat (16), we hypothesize that the spectrotemporal properties of natural acoustic stimuli evoke greater response selectivity than artificial sounds matched in bandwidth, duration, and amplitude.

Results

Audio recordings of echolocation call sequences taken from big brown bats tracking a moving target served as natural acoustic stimuli for neurophysiological experiments (Fig. 1A). Auditory responses of SC neurons to natural call sequences were compared with artificial sound sequences containing linear frequency-modulated (FM) and white noise elements matched in amplitude, duration, and bandwidth (Figs. 1B and C). Data were collected from five bats, resulting in 56 single-unit recordings and 127 multiunit recordings. All analyses presented below were performed on the single-unit responses to sounds broadcast through the loudspeaker direction

Significance

Nervous systems have evolved to enable processing of complex stimuli that animals encounter in their natural environments, and yet, neurophysiological research has largely investigated responses to simple artificial stimuli. In an attempt to bridge this gap, we characterized response selectivity to natural stimuli in the midbrain superior colliculus (SC) of the echolocating bat, an animal that probes the environment with sonar vocalizations. Using the bat's dynamic echolocation signals as auditory stimuli, we discovered that SC neurons in the dorsal sensory layers exhibited selectivity that was not predicted by responses to artificial sounds. Our research reveals how response properties of SC auditory neurons may lead to stimulus selection and further shows the importance of biologically relevant stimuli to understand brain function.

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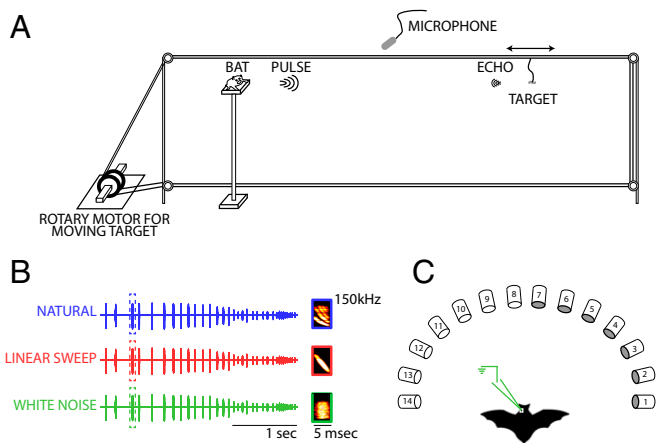


Fig. 1. (A) Behavioral setup for recording natural echolocation call approach sequences in the laboratory. (B) Three categories of sound stimuli: natural stimulus (blue), linear sweep stimulus (red), and white noise stimulus (green). Spectrograms in *Right* show the spectral properties of each stimulus. (C) The bat is awake and head-restrained at the center of an array of 14 speakers. Shaded speakers indicate stimulus presentations in the contralateral hemifield (green recording probe in the opposite hemisphere).

evoking a maximum response (Fig. 1C). Auditory unit responses to the brief stimulus elements were phasic (Fig. S1) and found across all recording depths (from 300 to 1,050 μm), showing that auditory activity can be elicited from neurons across the functional subdivisions in the bat SC.

Shown in Fig. 2A–C are example responses to each stimulus category (natural, linear sweep, and white noise in Fig. 2A–C, respectively) for a single neuron. SC neurons generally had very low levels of spontaneous activity and were typically active only in response to acoustic stimulation. Auditory activity evoked by each of three stimulus sets included a large onset response to the first element followed by time-locked responses to individual elements in the stimulus sequence. Response latencies varied significantly across the stimulus categories. The shortest latency responses were elicited by white noise stimuli followed by natural stimuli, with the longest latencies to linear sweep stimuli (Fig. S2) (Wilcoxon sign rank test; $P < 0.0001$ for all comparisons).

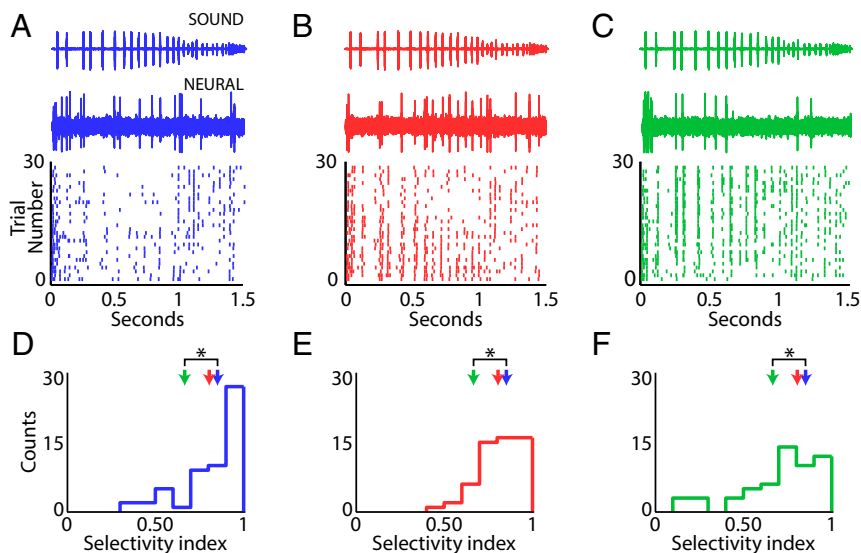


Fig. 2. (A, *Top*) Oscillogram of natural stimulus (blue). (A, *Middle*) One example of raw neural response to the natural stimulus. (A, *Bottom*) Raster plot of 30 trials of the natural stimulus presentation. (B) The same as in A but for the linear sweep stimulus (red). (C) The same as in A but for the white noise stimulus (green). (D–F) Auditory response selectivity across (D) natural (blue), (E) linear sweep (red), and (F) white noise stimuli (green). (D) The natural stimulus evoked the highest selectivity (mean = 0.817) and was significantly greater than selectivity to (F) the white noise stimulus (mean = 0.71; Kolmogorov–Smirnov test; $P = 0.03$) but not (E) the linear sweep response selectivity (mean = 0.807; KS test; $P = 0.20$). Differences in response selectivity between the linear sweep stimulus and the white noise stimulus were also not significant (KS test; $P = 0.20$). Arrows on each histogram represent mean values.

SC neurons also showed differences in auditory response selectivity across stimulus categories. Response selectivity was higher for spectrotemporally structured stimuli than for the white noise stimuli. For example, the neuron shown in Fig. 2D–F responds to fewer individual sound elements in the natural and linear sweep stimuli than the white noise stimulus, respectively. Moreover, the patterns of responses (i.e., which sound elements elicited a response) were also different between the three stimulus categories. Across all recording sites, neural responses to the natural stimulus showed the highest stimulus selectivity (Fig. 2D, blue line) (mean selectivity index = 0.817) followed by responses to the linear sweep stimulus (Fig. 2E, red line) (mean selectivity index = 0.807) and responses to the white noise stimulus (Fig. 2F, green line) (mean selectivity index = 0.713). For all sites, the mean response selectivity of the natural stimulus was significantly greater than response selectivity to the white noise stimuli [Kolmogorov–Smirnov (KS) test; $P = 0.03$], but no other pairwise comparisons yielded significant differences. Interestingly, we found that auditory selectivity differed across recording depths. At more dorsal locations, auditory selectivity was found to be higher in response to the natural stimuli than the linear sweep or white noise stimuli (Fig. S3, dorsal) (permutation test; $P < 0.001$). In ventral SC, however, auditory response selectivity was comparable across stimuli (Fig. S3, ventral) (permutation test; $P > 0.05$). A more detailed analysis of the differences in selectivity throughout the layers of the SC is presented below.

Changes in auditory selectivity for each stimulus set category from dorsal to ventral SC are revealed by arranging neural responses in order of increasing recording depth. Normalized activity heat plots for all auditory responses to a set of the natural (Fig. 3A), linear sweep (Fig. 3B), and white noise stimuli (Fig. 3C) show a gradual decrease in selectivity with recording depth. Data from the same neuron are displayed along the same row in each panel of the activity heat plots for the three stimulus sets; red indicates a large evoked response, and blue indicates no response (Fig. 3). Responses to the natural stimulus (Fig. 3A) show a pronounced decrease in response selectivity as a function of increasing recording depth: fewer individual sonar pulses of the natural stimulus evoke responses in dorsal layers compared with neurons in ventral layers. SC responses to the linear sweep stimulus also exhibit a decrease in auditory response selectivity with increasing recording depths (Fig. 3B), but responses to the white noise stimulus set show little selectivity, with robust responses at both dorsal and ventral recording sites (Fig. 3C).

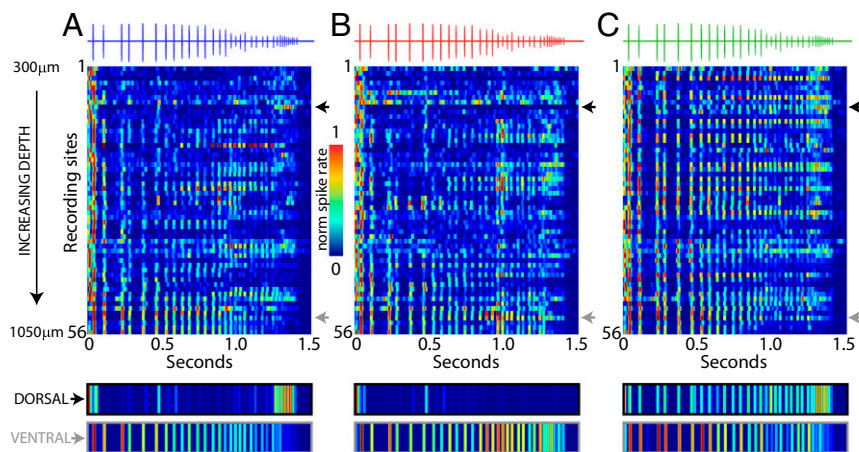


Fig. 3. (A, Upper) Oscillogram of the natural stimulus (blue). (A, Lower) Heat plot of normalized responses to the natural stimulus for all single units sorted by increasing depth (from top to bottom of the plot). Arrows denote neurons that are shown in greater detail below. (B, Upper) Oscillogram and (B, Lower) normalized heat plot of responses to the linear sweep stimulus (red). (C, Upper) Oscillogram and (C, Lower) normalized heat plot of responses to the white noise stimulus (green).

The black and gray arrows in Fig. 3 mark data recorded at dorsal and ventral locations, respectively, which are shown in greater detail in Fig. S4.

A quantification of the effect of recording depth on auditory response selectivity is presented in Fig. 4. Response selectivity was defined as the inverse proportion of the sound elements in a stimulus evoking a response greater than the half-maximum response. Using this metric, there is a significant correlation between recording depth and response selectivity to the natural stimuli (Fig. 4A) ($r = -0.49$; $r^2 = 0.24$; $P < 0.0001$). A similar trend is found for the responses to the linear sweep stimuli (Fig. 4B) ($r = -0.36$; $r^2 = 0.13$; $P = 0.006$), but selectivity in the responses to the white noise stimulus did not change with the depth of recording (Fig. 4C) ($r = 0.13$; $P = 0.35$). The white noise stimulus was an effective stimulus for evoking auditory responses throughout the bat SC, and as a result, responses to a larger number of sound elements in each white noise stimulus set were found across all layers, with little change in selectivity at different recording depths.

Given that auditory response similarity across stimulus categories varies with recording depth, we investigated whether neural selectivity to the underlying spectral features of the stimuli was driving the differences in responses. For this analysis, we determined the extent to which an SC neuron's response profile to the spectral features of the linear sweep stimulus set could be used to predict its response to the natural, multiharmonic, hyperbolic FM stimulus set. The white noise stimulus set was excluded from this analysis because of its lack of a well-defined spectrotemporal structure. Predictions for responses to the natural stimulus set were calculated using a generalized linear model (GLM) based on responses to the linear sweep stimulus set. The predictors for the GLM were five individual spectrotemporal features of each sound element as outlined in *Methods* (sonar pulse amplitude, duration, amplitude slope, spectral peak, and end frequency of the sweep), and the observed responses were the average auditory responses elicited by the linear sweep stimulus at each site. Shown in Fig. 5A and B are the data from one neuron located at a dorsal location in

the SC (384 μm from the SC surface). In Fig. 5A, *Middle*, the average responses of this neuron to the natural (blue) and linear sweep (red) stimuli are shown. Fig. 5A, *Bottom* displays the predicted response (black) to the natural stimulus as well as the observed natural stimulus response (blue). The predicted and observed responses are compared in Fig. 5B, and for this neuron, there is no significant relationship between the predicted and observed responses to the natural stimulus ($r = -0.29$; $P = 0.12$). Fig. 5C and D illustrates the results of a GLM analysis for a neuron recorded at a more ventral location in the SC (850 μm from the SC surface). The responses to the natural and linear sweep stimuli (Fig. 5C, *Middle*, blue and red lines, respectively) are more similar than those of the neuron shown in Fig. 5A; as a result, the GLM prediction of natural stimulus responses based on the linear sweep stimulus responses is better for this neuron. The efficacy of the GLM prediction can be seen qualitatively in the similarity of the predicted and observed responses in Fig. 5C, *Bottom* and quantitatively in Fig. 5D. Fig. 5D plots the predicted response against the observed response to the natural stimulus. The correlation coefficient for this comparison is highly significant ($r = 0.77$; $P < 0.0001$), showing that responses of this ventral SC neuron to the natural stimulus could be reliably predicted from responses to the linear sweep stimulus.

To more thoroughly examine how the depth of recording in SC influences the predictions of stimulus responses based on spectrotemporal features, a GLM was calculated for each neuron (as described above), and the stimulus response predictions were analyzed with respect to the depth of the recording location. Fig. 5E compares the recording depth of each neuron and how well the linear sweep stimulus responses predict natural stimulus responses by measuring changes in correlation coefficients (r values). For these data, there is a significant correlation between recording depth and the accuracy of the GLM prediction ($r = 0.71$; $P < 0.0001$). Moreover, the neurons for which the linear sweep-based GLM model was a reliable predictor of natural stimulus responses were clustered in the deeper recording sites

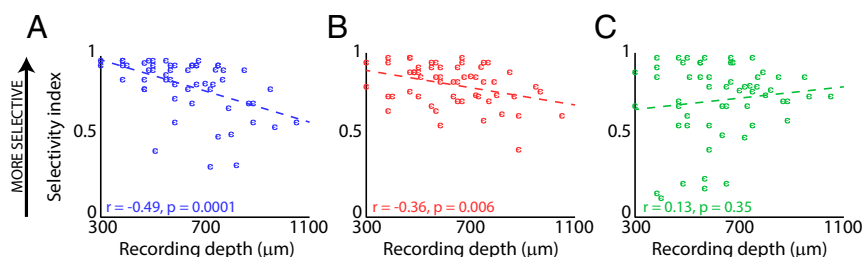


Fig. 4. (A) Response selectivity to the natural stimulus (blue) as a function of depth. There is a significant negative correlation between response selectivity and depth of recording site ($r = -0.49$; $P = 0.0001$; $r^2 = 0.24$). Selectivity increases along the y axis. (B) Response selectivity to the linear sweep stimulus (red) as a function of depth ($r = -0.36$; $P = 0.006$; $r^2 = 0.13$). (C) Response selectivity to the white noise stimulus (green) as a function of depth ($r = 0.13$; $P = 0.35$).

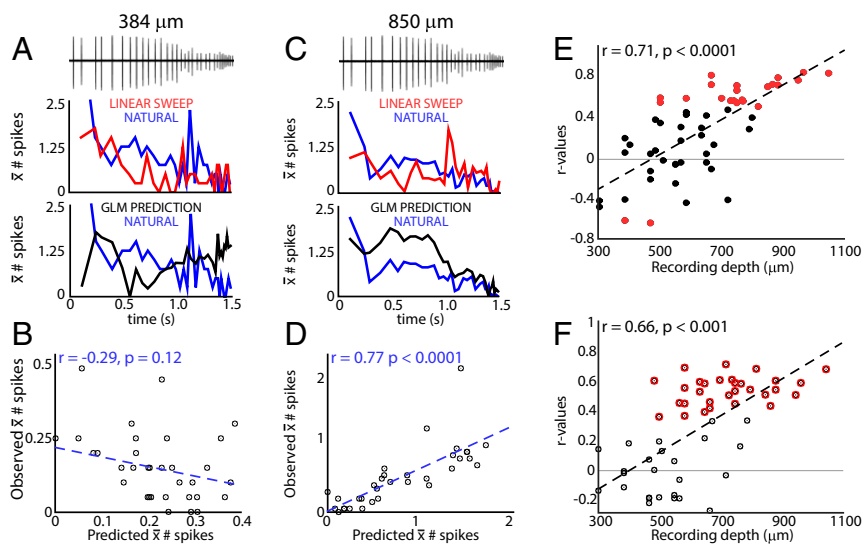


Fig. 5. (A) GLM fit from a dorsal recording location: 384 μm . (A, Top) Oscillogram of the stimulus. (A, Middle) Average spikes per trial for responses to the natural stimulus (blue) and the linear sweep stimulus (red). (A, Bottom) GLM-predicted responses to the natural stimulus (black) and the observed natural stimulus responses (blue). (B) There is no correlation between the GLM-predicted response and the observed natural stimulus response ($r = -0.29$; $P = 0.12$). (C) GLM fit from a ventral recording location: 850 μm (same as in A). (D) There is a significant correlation between the GLM-predicted response and the observed natural stimulus response ($r = 0.77$; $P < 0.0001$). (E) Significant relationship between recording depth and GLM fit correlations of observed and predicted natural stimulus responses ($r = 0.71$; $P < 0.0001$). Red circles indicate r values of significant correlation coefficients ($P < 0.0009$; with Bonferroni correction), and black circles are data with nonsignificant coefficients. (F) Relationship between recording depth and correlations between observed and GLM-predicted linear sweep responses based on natural stimulus response fitting ($r = 0.66$; $P < 0.001$). Colors are the same as in E.

(Fig. 5E, red data points indicate significant r values) ($P < 0.0009$ with Bonferroni correction). We were, however, concerned that the results of the GLM predictions may be dependent on the stimulus responses used to construct the GLM. To examine this possibility, we constructed GLMs based on the responses to the natural stimulus to predict responses to the linear sweep stimulus (i.e., reversing the direction of the GLM). The results for natural stimulus-based GLMs were consistent with those for GLMs constructed from linear sweep responses (Fig. 5F): the natural stimulus responses failed to predict linear sweep responses in dorsal SC, whereas in ventral SC, the natural stimulus responses were good predictors of linear sweep responses. Lastly, to rule out the effects of auditory response selectivity on GLM predictions, we compared response selectivity with GLM model accuracy (Fig. S5) and found no significant relationship.

Discussion

The midbrain SC has long been implicated in sensory grasp—for behaviors such as saccadic eye movements, pinnae movements, head movements, and body movements—pointing the primary sensory apparatus toward a salient stimulus (17–20). Sensory grasp not only requires stimulus localization but also, stimulus selection. Prior studies have examined the activity of SC/OT neurons related to visual stimulus selection (5, 8, 21, 22), but questions remain on how SC neurons differentially respond to acoustic stimuli to enable target selection for auditory grasp. The echolocating bat's active acoustic sensing system is particularly well-suited to investigate auditory response selectivity, because the very signals that the animal uses to track and intercept sonar targets can serve as acoustic stimuli in neural recordings to recreate natural listening experiences. The research findings reported here contribute to a broader understanding of SC function by showing auditory response selectivity of neurons in dorsal SC of the echolocating bat, an animal that relies on discriminating echo returns from its sonar vocalizations to orient and capture an auditory object in 3D space.

SC auditory selectivity was observed in this study to stimuli containing the natural spectrotemporal features of acoustic signals used by the bat for spatial orientation (i.e., sonar vocalizations), and this selectivity may serve to guide rapid species-specific orienting behaviors. Here, our data show clear differences in auditory response selectivity of dorsal SC neurons to the natural multiharmonic FM, linear FM sweep, and white noise stimulus sets matched in duration, bandwidth, and amplitude but differing in spectrotemporal structure. Previous research has shown that activity of SC neurons can be

modulated by the acoustic properties of sounds [e.g., binaural and monaural stimuli (23), apparent motion (24), and temporal separation between simulated sonar pulse and echo (10)]. SC neurons in these studies were selective to changes in the patterning of acoustic stimuli in either space or time. This study provides the first detailed examination, to our knowledge, of spectrotemporal acoustic selectivity of neurons across SC laminae, and this auditory selectivity is likely to underlie neural processes contributing to stimulus selection for orientation.

Possible sources of SC auditory selectivity are intrinsic and/or afferent inhibitory circuitry (25–27). In this study, neurons in dorsal SC showed greatest selectivity to FM stimuli, and previous research suggests that inhibitory interactions may underlie the sharpening of auditory responses to FM sweeps (28–30). Anatomically, prior work has shown that dorsal SC contains more lateral inhibitory connections than ventral SC (31). Furthermore, research on the visual subdivisions of the SC identified lateral inhibition that contributes to sensory selectivity (32), and additionally modeling studies have also suggested that intrinsic inhibitory mechanisms underlie stimulus selection in the SC (33). Additionally, projections from superficial layers to deeper layers are highly convergent (34), and such convergence results in the integration of activity over a large pool of differentially tuned sensory neurons, broadening the tuning of more ventral SC neurons. We hypothesize that the decreased response selectivity of deeper-layer SC neurons is a result of the merging of activity across superficial sensory neurons tuned to different spectrotemporal components of echo sound sources. In a manner similar to that in this study, deeper-layer SC neurons in the monkey were also found to be responsive to multiple features of a stimulus and even stimulation across multiple sensory modalities (35).

In terms of extrinsic connections, descending projections to the SC from auditory cortex and ascending projections from inferior colliculus have been shown to shape FM sweep selectivity (36, 37). Additionally, microstimulation of the forebrain in barn owls increases spatial selectivity in the OT (38), identifying another source of stimulus selectivity in midbrain neurons. In other work on the barn owl, temporally inactivating the nucleus isthmi pars magnocellularis, an inhibitory structure projecting to the OT, eliminated competitive stimulus interactions in the OT (39). Analogous inhibitory inputs to the SC, such as those originating in the substantia nigra (40, 41), may also contribute to the stimulus selectivity observed in this study. Lastly, cortical projections to the intermediate and deep layers of the SC are from multisensory areas of the cortex (42), suggesting that cortical

afferents convey a combination of signals to ventral SC and leading to larger response profiles. Selectivity to FM sweeps may, therefore, be imparted to SC neurons through projections from the inferior colliculus, auditory cortex, and other brain regions as well as generated intrinsically through lateral inhibitory connections.

Response selectivity arises through networks within sensory systems. In birds, for example, discrimination and identification of conspecific song require auditory processing to extract relevant acoustic features for social interactions (43). Previous research in the avian brain has identified multiple areas with neurons specifically tuned to the spectrotemporal features of bird song (43). Neural recordings from auditory areas of the avian brain reveal that tuning is dependent on the acoustic stimulus, with responses to artificial, simplified stimuli only approximating responses to the natural, acoustically complex song (44). In one study examining differences in auditory responses to complex, natural sounds and responses to simpler, artificially generated sounds, it was reported that auditory neurons exhibit extraclassical receptive fields arising through nonlinear tuning properties (13). We have also found nonlinear tuning properties in neurons found in the dorsal SC, and similar mechanisms are likely to operate in shaping auditory responses of SC neurons in this study. Our results motivate future research that assays SC function with stimulus parameters that are behaviorally relevant to the study animal to illuminate the natural processing of sensory stimuli for the purpose of selection.

Here, we report on auditory response selectivity in the bat SC, which holds relevance for this animal's natural behavior, namely the processing of echo returns to guide goal-directed orienting movements. We show that neurons in dorsal layers exhibit properties that support the selective processing of natural sensory signals, an important component of parsing and analyzing incoming sensory information (45) to enable rapid species-specific orienting behaviors (1). A lack of auditory selectivity in ventral locations of the bat SC may reflect the integration of auditory information to activate goal-directed orienting behaviors. These findings have implications for a broader understanding of the functional role of the midbrain in stimulus selection for sensory "grasp" across animal systems. Future research using ethologically relevant stimuli can further elucidate neural mechanisms for processing and attending to natural stimuli.

Methods

Big brown bats, *E. fuscus*, collected in the state of Maryland under a permit issued by the Department of Natural Resources served as subjects in behavioral and neurophysiological studies. The University of Maryland's Institutional Animal Care and Use Committee approved all of the procedures used for this study.

Behavioral studies yielded acoustic data for neurophysiological experiments. Three big brown bats were trained to rest on a platform and track a tethered insect, whose motion was controlled by a pulley system (Fig. 1A). The bat was rewarded at the end of each trial with the insect that it had tracked. Recordings were made of the bat's sonar vocalizations during insect approach with a Brüel and Kjær 1/8-in Calibrated Microphone and sampled at a rate of 500 kHz (National Instruments M-Series A/D Board; NI-DAQ) (example recording is shown in Fig. 1B, Top).

Five example recordings of echolocation call sequences taken from three bats were used to create a set of natural acoustic stimuli for neurophysiological experiments. In all recordings, the bats adaptively adjusted the time–frequency features of their vocalizations in response to prey location (46, 47). These recordings were then used as auditory stimuli and are referred to as the natural stimulus set.

We constructed a set of artificial stimuli with modifications to the spectrotemporal characteristics of the bats' sonar vocalizations (Fig. 1B). The first manipulation was a substitution of a single harmonic, linear FM down sweep for each multiharmonic, hyperbolic natural sonar vocalization. This stimulus is referred to as the linear sweep stimulus. The frequency range of the individual sound elements in the linear sweep stimulus was fixed at 20–100 kHz, matching the mean power of sonar pulses produced by bats tracking insects. Each sonar pulse in the natural stimulus was substituted with the artificially constructed linear FM sweep, maintaining the same time–amplitude profile as

the original recording. The second manipulation to the natural stimulus was a substitution of band-pass white noise for each recorded sonar pulse referred to as the white noise stimulus. The frequency band of the white noise stimulus was the same as the linear sweep stimulus (20–100 kHz) and also matched the time–amplitude profile of the natural stimulus. The linear sweep and white noise stimuli were designed to create acoustic stimuli with matching duration, bandwidth, amplitude, and repetition rate of the natural stimuli but differing in spectrotemporal parameters. Stimulus sets were based on five recorded echolocation sequences, yielding five natural, five linear sweep, and five white noise sequences (15 stimuli in total), each with individual variations in the timing and amplitude of signals. The stimuli were presented to the bat through an array of 14 custom-made electrostatic speakers (Fig. 1C). The peak amplitude of each stimulus set was 85-dB sound pressure level (SPL), but because of the amplitude changes in the natural stimulus, the amplitude of the signal elements within each set ranged from 78 to 85-dB SPL.

Extracellular recordings were taken from auditory neurons in the SC of five awake, passively listening bats. On the day of the experiment, the bat was placed in a custom holder, its head was fixed in position with a head post, and a craniotomy was performed over the SC. In the bat, the SC sits on the dorsal surface of the brain beneath two layers of skull. On the day of the head post surgery, the first layer of skull is removed. On the day of the experiment, a craniotomy is made with a small burr attached to a dental drill in the second layer of skull. Making the craniotomy in this fashion is very fast and precludes the need for anesthesia; therefore, no postanesthesia effects on neural responses were of concern during the day of the experiment. The recording device was a silicon probe from Neuronexus that had a 4 × 4 arrangement of recording sites (total size of 300- μ m wide and 375- μ m deep, 100 μ m between shanks, and 125- μ m spacing between recording sites on each shank). The order of the stimuli as well as the speaker broadcast position were randomized to avoid any possible effects of repeatedly presenting a stimulus from the same location. Auditory sites studied with at least 20 presentations of each stimulus category were analyzed.

The recorded extracellular potentials were analyzed using MATLAB 2012b. We first determined if the recordings were extracellular potentials of single neurons or multiple neurons. The single units were sorted by performing a principal components analysis on the spike waveforms (as described in ref. 48). The principal components analysis-based clustering resulted in single units that matched with qualitative assessments of spike waveforms and estimates of single-unit isolation based on spike refractory periods.

Auditory responses from the speaker location eliciting the largest response (number of spikes) were used for subsequent analysis (SI Methods and Fig. S6). The first analysis examined auditory response selectivity across three stimulus categories: natural, linear sweep, and white noise stimuli. Neural responses were analyzed in a 50-ms window starting at the onset of each sound element in a stimulus. When the interval between successive sound elements was less than 50 ms, spikes were counted only within the interval between the individual sound elements. The spike count in each response window was then converted into a spike rate value. For this study, we defined selectivity as the inverse proportion of the full stimulus response greater than or equal to the half-maximum response. As an example of this analysis, if a stimulus contained 30 individual sound elements, we determined which of those individual sound elements elicited the highest spike rate and then, calculated how many of the other 29 sound elements in a sequence elicited a response greater than or equal to one-half of the maximum response. If, for instance, 10 sound elements (these sound elements do not need to be consecutive in time for this analysis) in a 30 element stimulus sequence produce a response greater than or equal to one-half of the maximum response, the selectivity index would be 1.0–0.33 or 0.66. Other criteria were tested, such as examining responses greater than or equal to 25% or 33% of the maximum response, and these criteria generated similar results. The half-maximum response was ultimately chosen, because this threshold has been used in previous works to evaluate auditory tuning of SC neurons (49, 50). We also examined whether the speaker broadcast location affected response selectivity across the stimuli (Fig. S6) and found little to no effect.

In an effort to determine how well responses to one stimulus category predicted responses to a different stimulus category, a GLM built on linear sweep responses was used to calculate predicted natural stimulus responses. For each neuron recorded, the predictors (X) for the GLM were five of the spectral features outlined in SI Methods: the amplitude, duration, amplitude slope, spectral peak, and end frequency of the sweep. An iterative process was used to determine which of 10 total spectrotemporal features to include in constructing the GLM. All combinations between 2 and 10 features were tested to determine how well the GLM calculated from the linear sweep responses predicted the observed natural stimulus responses. The different feature combinations were tested by performing a cross-correlation between

the predicted responses and the observed responses. The observed response input to the GLM was the mean spike count for each sound element in the linear sweep stimulus, excluding responses to the first sound element. Responses to the very first sound element in each stimulus set were often onset responses that did not depend on the stimulus components. The predicted responses generated by the GLM were then compared with the observed responses by computing a correlation coefficient (r). Lastly, to examine whether the results of GLM fitting were dependent on the stimulus parameters used to construct the GLM, a GLM model based on natural stimulus responses was used to predict responses to the linear sweep stimulus.

This analysis was done in a similar fashion to that described above for linear sweep stimulus response predicting natural stimulus responses using a GLM.

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