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RESEARCH ARTICLE | *Sensory Processing*

Natural echolocation sequences evoke echo-delay selectivity in the auditory midbrain of the FM bat, *Eptesicus fuscus*

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Macías S, Luo J, Moss CF. Natural echolocation sequences evoke echo-delay selectivity in the auditory midbrain of the FM bat, *Eptesicus fuscus*. *J Neurophysiol* 120: 1323–1339, 2018. First published June 20, 2018; doi:10.1152/jn.00160.2018.—Echolocating bats must process temporal streams of sonar sounds to represent objects along the range axis. Neuronal echo-delay tuning, the putative mechanism of sonar ranging, has been characterized in the inferior colliculus (IC) of the mustached bat, an insectivorous species that produces echolocation calls consisting of constant frequency and frequency modulated (FM) components, but not in species that use FM signals alone. This raises questions about the mechanisms that give rise to echo-delay tuning in insectivorous bats that use different signal designs. To investigate whether stimulus context may account for species differences in echo-delay selectivity, we characterized single-unit responses in the IC of awake passively listening FM bats, *Eptesicus fuscus*, to broadcasts of natural sonar call-echo sequences, which contained dynamic changes in signal duration, interval, spectrotemporal structure, and echo-delay. In *E. fuscus*, neural selectivity to call-echo delay emerges in a population of IC neurons when stimulated with call-echo pairs presented at intervals mimicking those in a natural sonar sequence. To determine whether echo-delay selectivity also depends on the spectrotemporal features of individual sounds within natural sonar sequences, we studied responses to computer-generated echolocation signals that controlled for call interval, duration, bandwidth, sweep rate, and echo-delay. A subpopulation of IC neurons responded selectively to the combination of the spectrotemporal structure of natural call-echo pairs and their temporal patterning within a dynamic sonar sequence. These new findings suggest that the FM bat's fine control over biosonar signal parameters may modulate IC neuronal selectivity to the dimension of echo-delay.

NEW & NOTEWORTHY Echolocating bats perform precise auditory temporal computations to estimate their distance to objects. Here, we report that response selectivity of neurons in the inferior colliculus of a frequency modulated bat to call-echo delay, or target range tuning, depends on the temporal patterning and spectrotemporal features of sound elements in a natural echolocation sequence. We suggest that echo responses to objects at different distances are gated by the bat's active control over the spectrotemporal patterning of its sonar emissions.

animal sonar; mammalian auditory system; midbrain; range-tuned neurons

INTRODUCTION

Temporal signal processing in the auditory system of humans is of central importance to the perception of speech, music, pitch, and sound source location (Lyon and Shamma 1996; Moore 2012; Shamma 2001; Slaney and Lyon 1993). In echolocating bats, temporal processing of acoustic signals lays the foundation for sonar scene representation (Moss and Surlykke 2001, 2010), and a deeper knowledge of this representation may uncover general principles of temporal signal processing in other animal species that rely on sound to guide natural behaviors.

Echolocating bats broadcast sonar signals and process acoustic information carried by returning echoes to guide behavioral decisions for spatial orientation (Griffin 1958; Moss and Surlykke 2010). Fundamental to biosonar imaging is the measurement of echo arrival time, the bat's cue for target distance (Simmons 1971, 1973; Simmons et al. 1979). The posited biological substrate of sonar ranging is echo-delay tuning of single neurons in the bat auditory pathway. Echo-delay tuned neurons show facilitated responses to pairs of sounds that simulate an echolocation call and sonar return, over restricted time delays and corresponding distances (Suga 2015). Delay-tuned neurons have been reported in central auditory brain regions of bat species that use both constant frequency (CF) and frequency modulated (FM) echolocation signals: the auditory cortex (Dear et al. 1993; Hechavarría et al. 2013a; O'Neill and Suga 1982; Schuller et al. 1991; Sullivan 1982), the medial geniculate body (Olsen and Suga 1991), the intertectal nucleus (Dear and Suga 1995; Feng et al. 1978), and the superior colliculus (Valentine and Moss 1997).

Studies of the bat IC reveal species differences in echo-delay tuning. Research on the mustached bat (*Pteronotus parnellii*), a species that produces echolocation calls consisting of CF-FM components, suggests that the response characteristic of echo-delay tuning first emerges at the level of inferior colliculus (IC) (Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999; Suga 2015). Surprisingly, echo-delay tuned neurons have not been identified in the IC of the big brown bat, a species that uses only FM signals to forage for insect prey, even though researchers have explicitly searched for this response characteristic (Feng 2011; Feng et al. 1978). The divergent reports on echo-delay tuning in the IC of CF-FM and FM insectivorous bats thus raises the question whether stimulus context differentially influences echo-delay tuning in bat species that use different sonar signal designs.

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Most studies of echo-delay tuned responses in the bat auditory system have employed artificial acoustic signals constructed to mimic elements of natural echolocation signals. This approach permits careful control over stimulus parameters but fails to capture the dynamic spectrotemporal parameters of natural sonar signals produced by bats as they forage and navigate their surroundings (Schnitzler and Kalko 2001; Simmons et al. 1979).

Researchers have reported neural response selectivity to combinations of acoustic parameters in pulse-echo pairs and changes in neural selectivity to sound duration, frequency, azimuth, and echo-delay with variations in stimulus repetition rate and amplitude (Bartenstein et al. 2014; Galazyuk et al. 2000; Jen et al. 2001; O'Neill and Suga 1982; Wu and Jen 1996; Zhou and Jen 2001). These findings point to the complexity of stimulus response selectivity in the bat auditory system and suggest that past studies using artificial sounds may have failed to reveal fundamental features of biosonar signal processing in the midbrain IC of the FM bat, including the dimension of echo-delay tuning.

A small number of studies have characterized responses of bat auditory neurons to natural echolocation sequences, mimicking those produced by bats approaching objects. In the FM fruit bat, *Carollia perspicillata*, responses to natural echolocation sequences differ between the auditory cortex (AC) and IC. In the dorsal AC of anesthetized *C. perspicillata*, neurons showed facilitated echo-delay tuned responses, and natural sonar sequences evoked sharpened neuronal selectivity to call-echo pairs compared with the presentation of isolated call-echo pairs with variable delays (Beetz et al. 2016). By contrast, neurons in the IC of this species responded to every element in the sonar sequence, showing somewhat greater responses to certain call-echo elements (Beetz et al. 2017). Sanderson and Simmons (2005) studied neural responses of IC neurons in the FM insectivorous bat, *Eptesicus fuscus*, to naturalistic echolocation prey capture sonar sequences, containing call-echo pairs that changed in duration, frequency content, and repetition rate. Their data show that changes in sonar call duration, repetition rate, and signal amplitude modulate the strength of neural responses, and their analysis revealed that registration of a sonar target could be represented by single-unit spike latencies. They reported that many neurons respond selectively to a segment of a dynamic echolocation sequence, but they did not specifically investigate the dimension of echo-delay tuning.

Here, we studied auditory responses of neurons in the IC of the FM big brown bat, *E. fuscus*, with a specific focus on echo-delay tuning. We hypothesize that dynamic changes in acoustic parameters of a natural echolocation stimulus influence the echo-delay tuning characteristics of a population of IC neurons in the FM bat, *E. fuscus*. To test this hypothesis, we studied neural responses in the bat IC to natural echolocation sequences constructed from acoustic signals recorded from big brown bats tracking a moving object and to artificial stimuli that permitted independent control over stimulus repetition rate, duration, bandwidth, and sweep rate. Our results demonstrate that a population of neurons in the IC of the big brown bat shows response selectivity to the dimension of echo-delay, which is sensitive to the interval and spectrotemporal features of acoustic stimuli.

METHODS

Animals

Electrophysiological recordings from the IC were performed in six adult (2 males, 4 females) big brown bats, *E. fuscus*. Bats, collected in the state of Maryland under a permit issued by the Department of Natural Resources (Collecting permit no. 55440), were used as subjects in behavioral and neurophysiological studies. The Johns Hopkins University's Institutional Animal Care and Use Committee approved all procedures of this study.

Acoustic Stimuli

Both artificial and natural sounds served as stimuli in neural recording experiments. Pure tones of 5-ms duration (0.5-ms rise-fall times) and frequencies between 20 and 90 kHz (5-kHz steps) were broadcast at a constant sound pressure level at 70 dB sound pressure level (SPL), corrected for the loudspeaker frequency response. Each sound frequency was randomly presented 20 times with a 300-ms interstimulus interval.

Natural echolocation sequences were recorded from two big brown bats trained to rest on a platform and to track a moving target (a 9-cm-diameter Styrofoam sphere), whose motion was computer-controlled by a programmable pulley system. The target approached the bat at a speed of 3 m/s, which falls within the natural flight speed of the big brown bat (2–6 m/s; Falk et al. 2014). The calls that the bat emitted while tracking the Styrofoam target from the platform were recorded using a calibrated microphone (Brüel and Kjær 1/8-inch 4138), with a flat frequency ($\pm$0.5 dB) response up to 100 kHz, which was mounted perpendicularly at a distance of 10 cm in front of the bat (M1, Fig. 1*A*). Echoes returning from the target after each call were recorded with a second ultrasonic microphone (UM3; Ultra Sound Advice) mounted beneath the platform and was pointed at the target (M2, Fig. 1*A*). Both call (M1) and echo (M2) microphone signals were sampled at 250 kHz (PXIe 8135 controller with two PXIe 6358 data acquisition cards; National Instruments). In experiments, the broadcast signals were digitally high-pass filtered at 10 kHz (Butterworth filter, 6 order). To create a call-only sequence, echoes in the recordings from M1 were replaced by background noise recorded before the preceding call. Similarly, we created an echo-only sequence by replacing the calls with background noise using recordings from M2. After these sequences were created, the background noise level (based on root-mean-square calculations) in both recordings was matched by adjusting the stronger signal recordings (M1) to the weaker recordings (M2). The echolocation sequences used as acoustical stimulus in the neural recording experiments were then created by combining the calls and echoes from the processed "pure" M1 and M2 signals. In these stimulus sequences, each echolocation call was followed by an echo, with a target distance-dependent time delay (referred to as call-echo delay).

Sonar sequences. In the present study, two representative echolocation sequences, constructed from sound recordings of bats tracking the target from a platform, were used as acoustic stimuli for electrophysiological recording experiments. The first sequence (*sequence 1*; Fig. 1*B*) consisted of 30 stimulus elements, with each element containing a call-echo pair (Fig. 1, *C* and *D*), and echo delay reflecting target distance. In this sequence, call-echo delays decreased from 10.7 to 1.1 ms, which correspond to distance changes from 184 to 19 cm (Fig. 1*E*), and the duration of calls decreased from around 1.9 to 0.7 ms (Fig. 1*F*). During auditory stimulation, the maximum sound level reaching the bat's ears was an echolocation call at 70 dB SPL (peak amplitude). The amplitude of sound elements in the playback sequences was determined with a measurement microphone (Brüel and Kjær 1/8-inch 4138) placed at the same distance (i.e., 60 cm) as the custom loudspeaker (1-cm-diaphragm electrostatic speaker) that broadcast stimuli in the experiment. The sensitivity of the 1/8-inch measurement microphone was determined with a sound calibrator

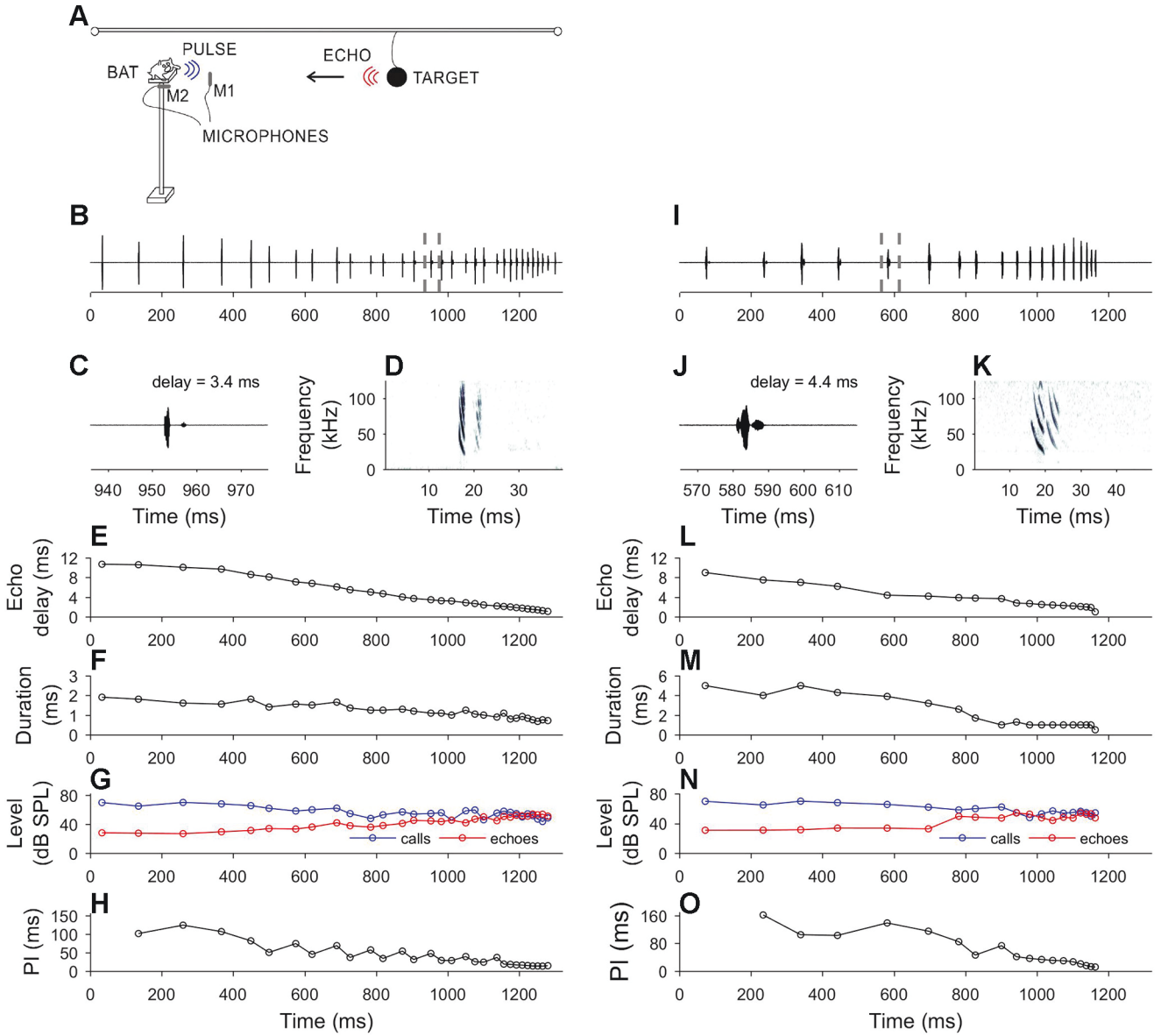

Fig. 1. Echolocation sequences used for auditory stimulation. *A*: behavioral setup for recording natural echolocation call sequences in the laboratory. M1 represents the microphone used to record the bats' vocalizations. M2 represents the microphone used to record the echoes coming from the target. *B*: oscillogram of *sequence 1* used for auditory stimulation. *C*: detailed oscillogram of a call-echo pair bracketed by broken lines in *B*. *D*: spectrogram of the example call-echo pair shown in *C*. *E*: variation of call-echo delay along echolocation *sequence 1*. *F*: variation of call duration along echolocation *sequence 1*. *G*: variation of call and echo level in *sequence 1*. *H*: variation of pulse interval in *sequence 1*. PI, pulse interval. *I*: oscillogram of *sequence 2* used for auditory stimulation. *J*: detailed oscillogram of a call-echo pair bracketed by broken lines in *I*. *K*: spectrogram of the example call-echo pair shown in *B*. *L*: variation of call-echo delay along echolocation *sequence 2*. *M*: variation of call duration along echolocation *sequence 2*. *N*: variation of call and echo level in *sequence 2*. *O*: variation of pulse interval in *sequence 2*.

(Brüel and Kjær 4231) that broadcast a constant 1-kHz pure tone at an amplitude of 94 dB SPL. To estimate the amplitude of all the calls and echoes in the sequence, we first calculated the ratios of their peak amplitude to the highest SPL call. Because this call was presented at amplitude of 70 dB SPL, the amplitude of all other calls and echoes can be estimated accordingly using the ratio values. SPL estimates of calls in *sequence 1* at the bat's ears ranged between 58 and 70 dB SPL, and echoes ranged between 28 and 54 dB SPL (Fig. 1*G*). Pulse interval (PI) decreased from 124 to 14 ms (Fig. 1*H*).

A total of 382 units were studied with *sequence 1*. To corroborate that characteristics of the neuronal responses were not restricted to a single echolocation sequence, nine units were also studied with a second echolocation sequence (*sequence 2*, Fig. 1, *I–O*) consisting of 19 call-echo pairs, and data were compared with responses to *sequence 1*. In *sequence 2* call-echo delays decreased from 9 to 1 ms, and duration of calls changed from 5 to 1 ms. Levels of calls ranged between 58 and 70 dB SPL, and level of echoes ranged between 30 and 54 dB SPL. Pulse interval decreased from 162 to 12 ms.

To investigate whether neural responses to elements in the natural sonar sequence depended on the combination of call-echo pairs, the following two new sequences were created from *sequence 1*: *1*) a "call only sequence" in which the echoes were removed [in the call only sequence the timing of the call stimuli (onset time and duration) was identical to timing of the calls in *sequence 1*]; and *2*) an "echo only sequence" in which the calls were removed (the timing of the echoes in echo only sequence was identical to timing of echo stimuli in *sequence 1*). The activity of 205 units in response to *sequence 1* and to the call only sequence and the echo only sequence is reported.

To investigate the contribution of stimulus history on neural responses, three additional stimuli were created. *1*) *Sequence 1* was divided into 30 segments, and each segment consisted of a call and its corresponding echo. Single segments were presented at the same relative sound pressure level as in the complete sequence. The response of 165 units to these 30 stimuli, together with *sequence 1*, randomly presented is described. We refer to this stimulus configuration as the "isolated call-echo pair condition," in contrast to the "natural call-echo pair condition," in which the *sequence 1* was played in its original form. *2*) In addition, the responses of 56 units were studied to a sequence in which the order of each call-echo pair was shuffled randomly but still preserved the call-echo intervals of natural *sequence 1*. *3*) We studied 56 units with a time-reversed natural sequence such that call-echo elements of *sequence 1* with shorter delays were presented first. In this condition, the individual call-echo elements were preserved in their original form such that the call still preceded the echo, and both were natural downward frequency-modulated signals.

The effect of the pulse interval on the neuronal response was investigated. For this, we studied 54 units using *sequence 1* and two additional stimulus sequences with different PIs (50 and 250 ms, respectively). Both new sequences maintained the same order of call-echo presentation as in the natural sequence.

The effect of the spectrotemporal characteristics of calls and echoes on IC neural responses was tested using additional stimulus sequences. The neuronal responses of 31 units to *sequence 1* were compared with responses when stimulated with an artificial sequence, consisting of computer-generated logarithmic FM sweeps with constant bandwidth of 80 kHz (between 20 and 100 kHz). Timing and duration of calls and echoes matched those in *sequence 1*. This sequence is referred as "Artificial FM Natural Duration." To assess the effect of the changing call and echo durations within the natural sequence, we studied the response of 31 units to *sequence 1* and two additional artificial sound sequences as follows: *1*) computer-generated logarithmic FM sweep sequence, with constant bandwidth (80 kHz) of calls and echoes and a fixed duration of 3 ms ("Artificial FM 3 ms") and *2*) a computer-generated logarithmic FM sweep sequence with constant bandwidth of constant calls and echoes a fixed duration of 1 ms ("Artificial FM 1 ms").

In neurophysiological recording experiments, acoustic stimuli were generated at a sampling rate of 250 kHz with a National Instruments card (PXIe 6358). The audio signal was transferred to an audio amplifier (Krohn-Hite 7500). The acoustic stimuli were broadcast with a calibrated custom-built ultrasonic loudspeaker located 60 cm from the bat's ear. We obtained a flat frequency response of the loudspeaker ($\pm$1 dB) through digitally filtering the playback stimuli with the inverse impulse response of the playback system (Luo and Moss 2017). In each stimulus set, the order of presentation of the acoustic stimuli was randomized, and the interstimulus interval was 300 ms. We analyzed responses across 382 auditory sites with 20 presentations of each stimulus category.

Electrophysiological Recordings

Surgical preparation. Under 1–3% isofluorane gas anesthesia, the skin and temporal muscles overlying the skull were cut and removed, and a custom-fabricated post was attached to the bone at the midline using cyanoacrylate gel. Following surgery, Metacam (12.5 mg/kg) was administered orally for 2 days. To prevent infection, Panalog cream was applied to the muscles/skin of the surgical site, and 0.15 ml of Bactrim was injected one time daily. Bats were allowed to recover for a minimum of 2 days before neurophysiological recordings began.

Neural recording methods. Electrophysiological recordings were conducted in a sound-attenuating and electrically shielded chamber (Industrial Acoustics). Individually, bats were placed in a body mold made of plastic foam, and the head was tightly fixed by a rod attached to a metal holder. Each bat was used in multiple recording sessions (between 4 and 5 recording sessions/bat) that lasted a maximum of 4 h. No sedative or any other drugs were administered during recording sessions, and the bat rested quietly in the holder. With the use of skull and brain-surface landmarks, a small hole (of $\leq$1 mm diameter) was made over the IC with a scalpel blade. Neuronal recordings were performed using silicon probes from Neuronexus (a 1 $\times$ 16 probe with 50-μm spacing between recording sites and a 4 $\times$ 4 probe with 100 μm between shanks and 125-μm spacing between recording sites along each shank). Each shank had a thickness of 15 μm. The probe was advanced orthogonally to the brain surface, through intact dura mater. Recording depths were measured with a hydraulic microdrive (Stoelting) mounted on a micromanipulator. The brain surface was used as a reference point (0 μm) for depth measurement, and the recording depths ranged from 100 to 1,510 μm. A silver wire, placed 1–2 cm rostral to the recording electrode and underneath the skin, was used as grounding electrode. Neuronal data were acquired with an OmniPlex D Neural Data Acquisition System recording system (Plexon) at a sampling rate of 40 kHz (per channel) and 16-bit precision. Synchronization between the neural recordings and acoustic stimulus broadcasts was achieved with a TTL pulse output from the National Instrument card and recorded on one of the analog channels of the Plexon data acquisition system.

Analysis of Neuronal Recordings

Spike events were detected with a unit-specific threshold that was based on the spike amplitude relative to recording noise level. Recordings with spike amplitudes lower than four times the amplitude of the recording background noise were not included in the data analysis. Thus, the signal-to-noise ratio of the analyzed spikes was $\geq$12 dB. Neural recordings were sorted following methods outlined by Quiroga et al. (2004). The Wavelet transformation and the superparamagnetic clustering resulted in isolation of single-unit extracellular potentials that matched with qualitative assessments of spike waveforms and estimates of single-unit isolation based on spike refractory periods. Recordings with spike amplitudes lower than four times the amplitude of the recording background noise were not included in the data analysis. The best frequency (BF), which represents the frequency to which the neuron showed the highest number of spikes at 70 dB SPL, was measured for 109 units. Neuronal responses to all experimental conditions were visualized with dot raster displays and poststimulus time histograms (PSTH, 1-ms bin width). For each element in each stimulus, the neuronal response was quantified by measuring the number of spikes 5 ms before and 5 ms after the peak of the PSTH calculated in response to that particular element. A neuron was considered to be responsive to a particular element within any of the tested stimuli if the peak of the PSTH was four times higher than any peak in absence of the stimulus (Beetz et al. 2016).

In the responses of every neuron to each of the tested set of stimuli, we calculated a selectivity index as 1$-$ (no. of elements eliciting response/30). From the 30 call-echo pairs in *sequence 1*, we determined which of those pairs elicited a response. If, for instance, 10 call-echo pairs in a 30-element stimulus sequence elicited a response, the selectivity index would be 0.66 (= 1 – 10/30). To consider if a unit was selective, we set an arbitrary threshold selectivity index of 0.33, that is, a unit was considered to respond selectively to a specific group of call-echo pairs if it showed a response to 20 or fewer of the 30 call-echo pairs within *sequence 1*. For neurons showing selectivity to echo delay, we identified the best delay, i.e., the time separation between a call and its corresponding echo, which evoked the strongest response.

RESULTS

Extracellular recordings were obtained from 382 auditory units in the IC of six (2 males, 4 females) awake big brown bats, *E. fuscus*, using 16-channel silicon probes.

Tonotopic Organization and Frequency Representation

Single neuron responses were isolated at depths between 50 and 1500 μm. In 109 units, we determined each unit's BF based on its isolevel frequency response curve at 70 dB SPL. The BF represents the frequency to which the unit showed the highest number of spikes. A Spearman correlation analysis performed on data from 109 units showed that the BF increased with increasing recording depth ($R = 0.45$; $P < 0.05$, Fig. 2*A*, data plotted in gray), which is characteristic of the central IC (Casseday and Covey 1992; Jen and Schlegel 1982; Pinheiro et al. 1991). This tonotopic organization was evident across recordings. BF data across recording depths in one recording session from one animal are highlighted in red in Fig. 2*A*. The distribution of BFs measured in these 109 units showed a range between 20 and 85 kHz, with an overrepresentation of BFs between 25 and 30 kHz (Fig. 2*B*).

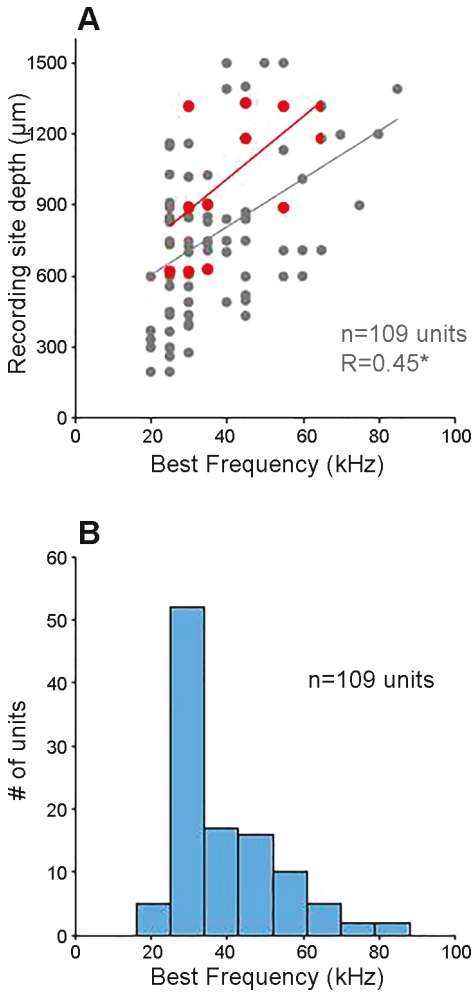

Fig. 2. Tonotopy and frequency representation of the inferior colliculus (IC) in *Eptesicus fuscus*. *A*: scatter plot showing the increase of the constant frequency (CF) along the recoding depth for 109 inferior colliculus units (gray dots). The number of units and the correlation coefficient (*R*) are given. Red dots and red line indicate relationship between best frequency and recording site depth of a single electrode penetration during a recording session in one animal. *B*: histogram representing the distribution of best frequencies (bin width = 5 kHz).

Natural Sonar Sequences Evoke Neural Echo Delay Tuning

Based on responses to *sequence 1*, neurons were classified as showing one of two distinct characteristics. One group of neurons showed no selectivity to stimuli within the sequence; 47% (177 units) of all recorded units responded to all call-echo pairs. The mean selectivity index calculated in this group of neurons was 0.042 ± 0.004. An example response is depicted in Fig. 3*A*. This unit responded to each of the call-echo pairs within the echolocation sequence, as well as to each of the acoustic elements in the echo only (Fig. 3*B*) and call only (Fig. 3*C*) sequences. This demonstrates that the presentation of the call-echo combination was not necessary to evoke a response in nonselective IC neurons. We found no significant differences when comparing the selectivity index calculated for each of the three conditions (Fig. 3*D*, Wilcoxon signed-rank test, $P = 0.17$).

A second group of neurons (53%, 205 units) responded only to restricted elements within the sequence, showing selectivity to a specific subset of call-echo pairs (Fig. 4, *A–C*), with a mean selectivity index of 0.77 ± 0.07. All of the following analyses were carried out on data collected from the second group of neurons showing response selectivity to a restricted number of call-echo pairs. PSTHs, with a bin size of 1 ms and dot raster displays representing temporal response patterns, were plotted for each neuron. The response to each element (call-echo pair) within the sequence was quantified by measuring the number of spikes in a window 5 ms before and 5 ms after the peak of the PSTH in the interval between one call-echo pair and the following. Based on the spike counts, we constructed an echo-delay tuning curve (Fig. 4, *A–C*). For each of the neurons showing a selective response, we determined its best delay, the time separation between a call and the corresponding echo, which evoked the highest number of spikes. The distribution of best delays across the neurons sampled is shown in Fig. 4*D*. Neurons responding to short call-echo delays (best delays <4 ms) comprised 73% of those studied. The distribution of echo-delay tuned neurons in our data from the IC of *Eptesicus* shows no topographic distribution of best delay along the dorsal-ventral axis (Fig. 4*E*).

Nine neurons were stimulated with a second echolocation sequence (*sequence 2*), and responses were compared with those obtained with *sequence 1*. The response of one of these nine neurons to these two echolocation sequences is shown in Fig. 5. When stimulated with *sequence 1* (Fig. 5, *A* and *B*), this neuron responded to call-echo delays between 2.8 and 5.03 ms (Fig. 5, *C* and *D*) and, when stimulated with *sequence 2*, to delays between 2.4 and 4.2 ms (Fig. 5, *F–H*). Best delays of neurons measured with the two separate echolocation sequences were not statistically different (Fig. 5*I*, Wilcoxon signed-rank test, $P = 0.10$).

To test if neural selectivity to elements in the natural sonar sequence depended on the combination of call-echo pairs, we created a call only sequence, where the echoes were removed, and an echo only sequence, where the calls were removed. In both, the timing of the sequences was unchanged. The responses of one neuron to these sequences are shown in Fig. 6. This neuron produced very few spikes in response to any sounds included in the call only sequence (Fig. 6, *A* and *B*) or to sounds in the echo only sequence (Fig. 6, *C* and *D*). By

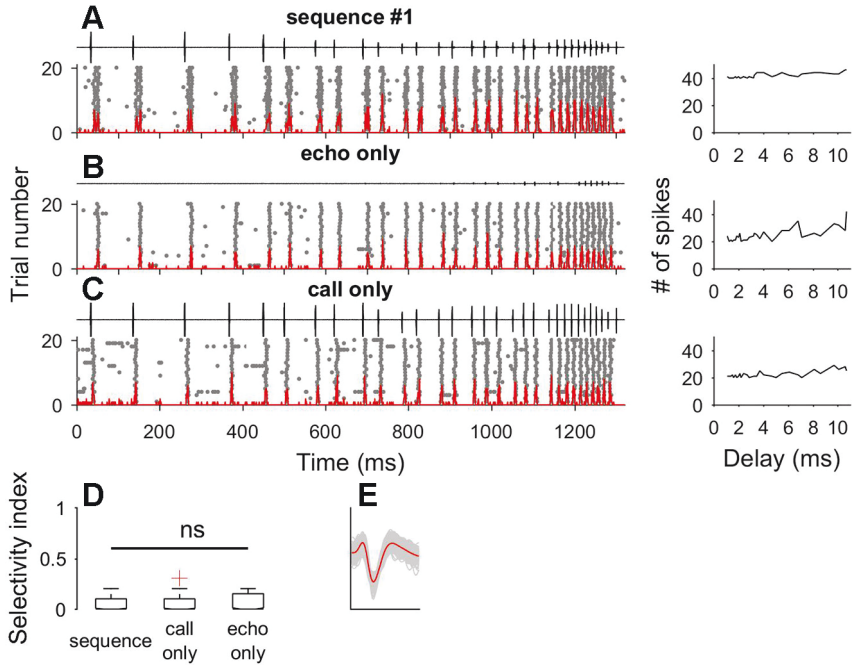

Fig. 3. Response of a nonselective neuron to natural echolocation sequence. *A*: response to *sequence 1*. *B*: response to the echo only sequence. *C*: response to the call only sequence. In each response is shown the acoustic stimulus, the dot raster display (gray dots), and poststimulus time histograms (PSTH, red line, 1-ms binwidth) of the response and the delay tuning curve. *D*: comparison of the selectivity index calculated in 177 units (Wilcoxon signed-rank test, $P = 0.17$). *E*: 2-ms segment of action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting.

contrast, Fig. 6, *E* and *F*, shows that the neuron was strongly facilitated by the combination of the calls and echoes, when the echo was delayed by 5.5–8.6 ms relative to the onset of the call (Fig. 6*G*). We quantified the magnitude of facilitation to call-echo pairs as a ratio, or facilitation index, with respect to the sum of responses to calls and echoes alone (Dear and Suga 1995; O'Neill and Suga 1982; Portfors and Wenstrup 1999; Yan and Suga 1996). The facilitation index was defined as (Rpe − Rp – Re)/(Rpe + Rp + Re) where Rpe, Rp, and Re are, respectively, the neuron's responses to the call-echo pair, call alone, and echo alone. A facilitation index larger than zero thus means that a neuron fires more spikes when stimulated with call-echo pairs than the sum of spikes elicited with call alone and echo alone. The facilitation index ranged from 0.09 to 1 at the neuron's best delay, with a mean of 0.30 ± 0.24 SD ($n = 205$ neurons, Fig. 6*H*). Following the criterion of previous studies (Portfors and Wenstrup 1999), a neuron is considered to exhibit call-echo delay facilitation if the index is ≥0.09, corresponding to an increase in response by 20% above the summed responses to the calls and echoes alone.

Selective Responses Occur After Echo Reception

Delay-tuned responses to specific call-echo pairs in the sequence were time locked to the echo offset. The response

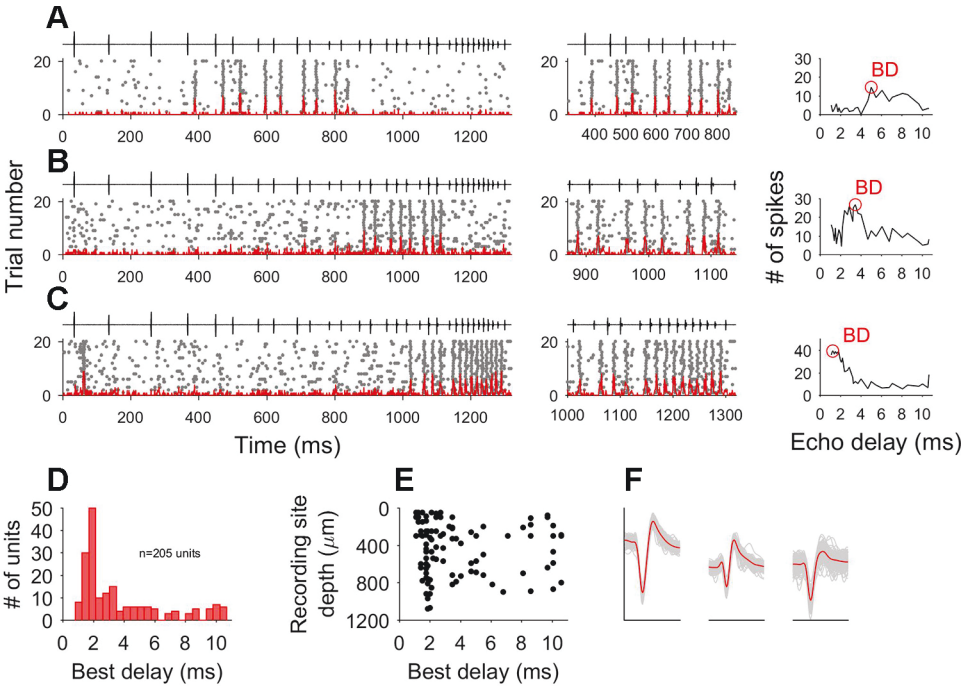

Fig. 4. Response of three units showing selectivity to a restricted subset of call-echo pairs. *A–C*: waveform of echolocation *sequence 1*, the dot raster display (gray dots), and poststimulus time histograms (PSTH, red line, 1-ms binwidth) of the response, a detailed view of the call-echo pairs evoking response and the corresponding unit activity and delay tuning curve. Best delay (BD) corresponds to the call-echo time separation eliciting the maximum response. *D*: distribution of best delays measured on the 205 neurons showing response selectivity to specific call-echo pairs (bin width = 0.5 ms). *E*: recording depth as a function of the best delay ($n = 205$ units). *F*: 2-ms segment of action potential waveforms obtained after spike sorting for these three units (*left*, unit shown in *A*; *center*, unit shown in *B*; *right*, unit shown in *C*).

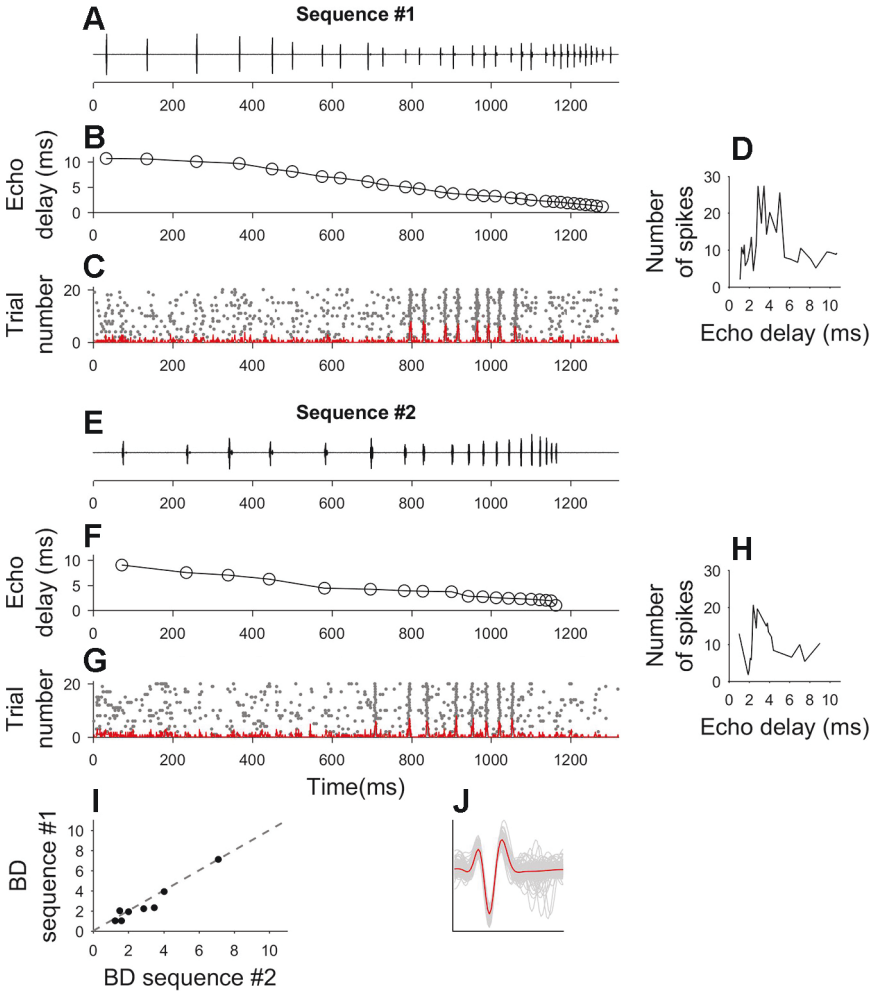

Fig. 5. Neuronal response to two different echolocation sequences. *A*: oscillogram of echolocation *sequence 1*. *B*: variation of echo delay in echolocation *sequence 1*. *C*: example of a unit response to echolocation *sequence 1*. *D*: delay tuning curve of the response shown in *C*. *E*: oscillogram of echolocation *sequence 2*. *F*: variation of echo delay along *sequence 2*. *G*: response of the same unit to *sequence 2*. *H*: delay tuning curve in response to *sequence 2*. *I*: comparison of best delays (BD) measured in the response of nine units to both echolocation sequences (Wilcoxon signed-rank test, $P = 0.10$). *J*: 2-ms segment of action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting.

of an example unit to *sequence 1* is shown in Fig. 7*A*. This neuron shows call-echo-delay tuning, with increased activity at echo delays between 3.2 and 4.7 ms (Fig. 7*B*). The latency of this neuron's response, measured as the time at which the PSTH reached 25% of its peak after call onset, increased with echo delay (Fig. 7*B*, circles), and the response latency from echo onset remained relatively constant (Fig. 7*B*, stars). To quantify the relation between response latencies to the call and echo of the paired stimuli, we computed separate linear regressions between call onset and echo onset and response latency in 205 neurons. The median slope of the curve for the latency after call onset was 1.05, indicating that the response latency of neurons increased linearly with increasing call-echo delay. By contrast, the median slope of the regression curve for latency after echo onset was −0.043, showing that the response latency of neurons was relatively constant with respect to echo reception time. The slopes of these call and echo response latency curves were significantly different (Wilcoxon signed-rank test, $P < 0.001$, Fig. 7*C*). We plot the call latency of the 1,010 facilitated response measured in 205 neurons against the call-echo delay that elicited that response (Fig. 7*D*). These latencies were always longer than the call-echo delay. This indicates that these responses were most likely locked to the echo arrival, instead of the call onset.

Response Selectivity Is Not Evoked by Isolated Call-Echo Pairs

We explored the contribution of stimulus context to neural response selectivity in 165 neurons. *Sequence 1* was split into 30 call-echo pairs and played randomly at 300-ms interstimulus time intervals. We refer to this stimulus configuration as the isolated call-echo pair condition, in contrast to the natural call-echo pair condition, in which the echolocation sequence was played in its original temporal pattern (Beetz et al. 2016). Figure 8 shows the responses of two neurons to the call-echo pairs in the natural sequence and to the isolated call-echo pair condition. The unit represented in Fig. 8*A* showed a delay-tuned response to delays between 1.1 and 2.5 ms in response to the echolocation sequence (see delay tuning curve in Fig. 8*C*). However, in response to the isolated call-echo pair condition, this neuron responded to all 30 elements and showed no stimulus selectivity (Fig. 8, *B* and *D*). A similar response pattern is also evident in the unit shown in Fig. 8, *F*–*I*. In responses to the isolated stimulus condition, the neuron showed a response to both call and echoes. This is more evident in neurons responding to call-echo pairs with delays between 10.7 and 6.8 ms, where the PSTH showed two peaks (Fig. 8, *B* and *G*). In these examples, each isolated and randomly presented call-echo pair elicited a strong response (~20 spikes). When call-echo pairs were presented within the

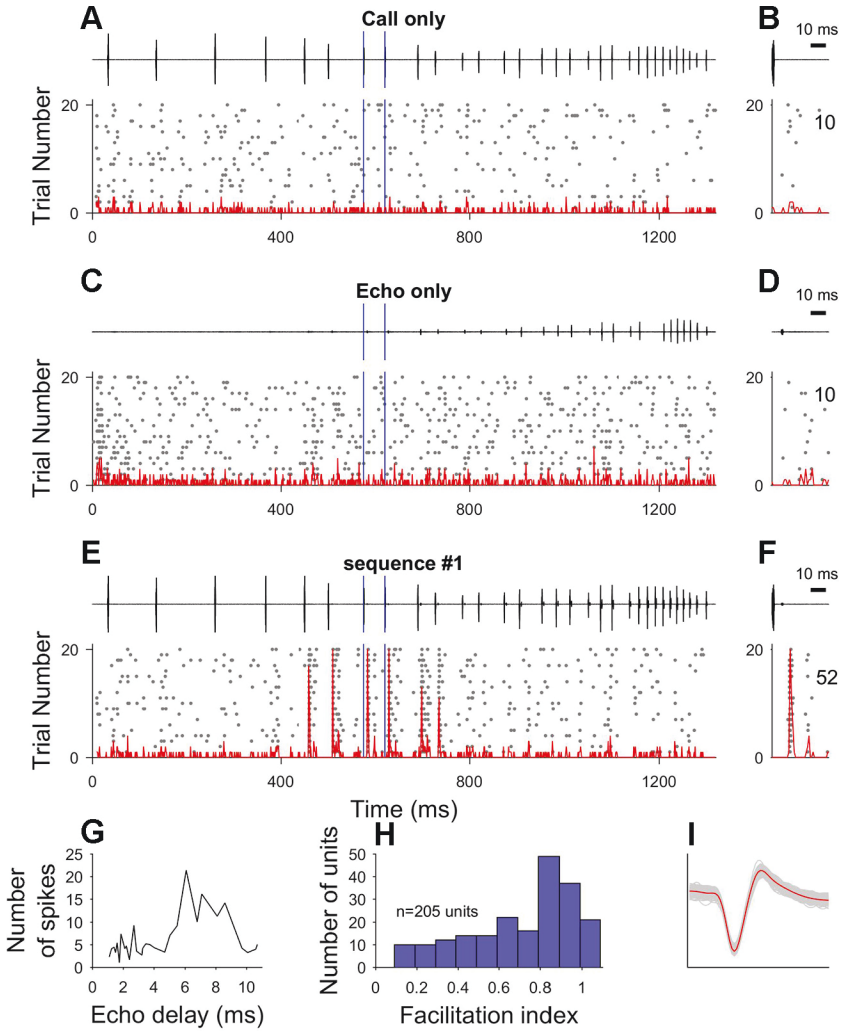

Fig. 6. Neuronal response of an IC neuron to call only, echo only, and call-echo *sequence 1*. *A*: response to call only sequence. *B*: detailed response to the specific call-echo pair highlighted between the two vertical blue lines in *A*. No. in the response indicates the no. of spikes. *C*: response to echo only sequence. *D*: detailed response to the specific call-echo pair highlighted between the two vertical blue lines in *C*. *E*: response to *sequence 1*. *F*: detailed response to the specific call-echo pair (delay = 6 ms) highlighted between the two vertical blue lines in *E*. Facilitation index for this unit is 0.44. *G*: delay-tuning curve of this unit, with a selective response to echo delays between 5.5 and 8.6 ms. *H*: facilitation index calculated in the neuronal population (n = 205 units, bin width = 0.1). *I*: 2-ms segment of action potential waveforms (y-axis in arbitrary units) obtained after spike sorting.

sequence, the first call-echo pair evoked no response in 46% of the sampled neurons (76 units), whereas, in the remaining 54% (89 units), the response to the first call-echo pair was similar to the response to the corresponding isolated pair. The mechanism underlying this result is not understood; however, Sanderson and Simmons (2005) also reported that a number of IC neurons in *E. fuscus* did not respond to the first call-echo pair in a sequence. The mean selectivity index of 165 neurons to the

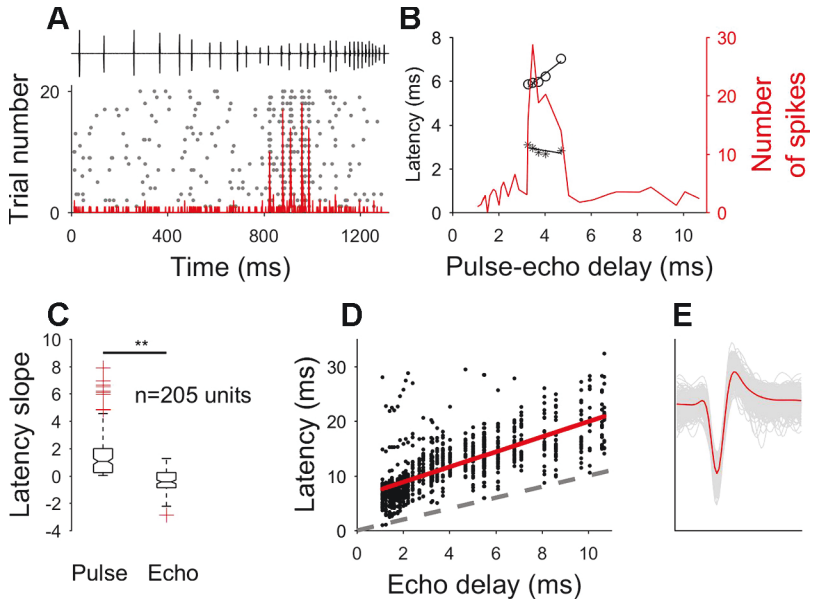

Fig. 7. Response latency to specific call-echo pairs in natural sequence. *A*: example of a neuron responding to specific call-echo pairs in the natural sequence [*top*, oscillogram of the pulse only sequence; *bottom*, dot raster display (gray dots) and poststimulus time histograms (PSTH, red line, 1-ms bin) of the response]. *B*: delay-tuning curve of this unit. Included is the relationship between call-echo delay and latencies after pulse onset (circles) and echo onset (stars) measured in the responses shown in *A*. *C*: comparison of the slope of the regression curve between delay and latencies calculated for the 205 neurons. Stars represent significant differences after Wilcoxon signed-rank test (**$P < 0.001$). Box-whisker plots show the median (50th percentile) as a solid red line inside the box delimited by the 25th and 75th percentiles with whiskers extending to the 10th and 90th percentile. Outliers are plotted individually (i.e., red crosses). *D*: latency measured after pulse onset for every response obtained in all delay-tuned neurons. Red line represents the regression curve. Gray line represents the echo delay. (Pearson correlation, n = 1,010 responses, R = 0.71). *E*: 2-ms segment of overlapping action potential waveforms (y-axis in arbitrary units) obtained after spike sorting of the unit shown in *A*.

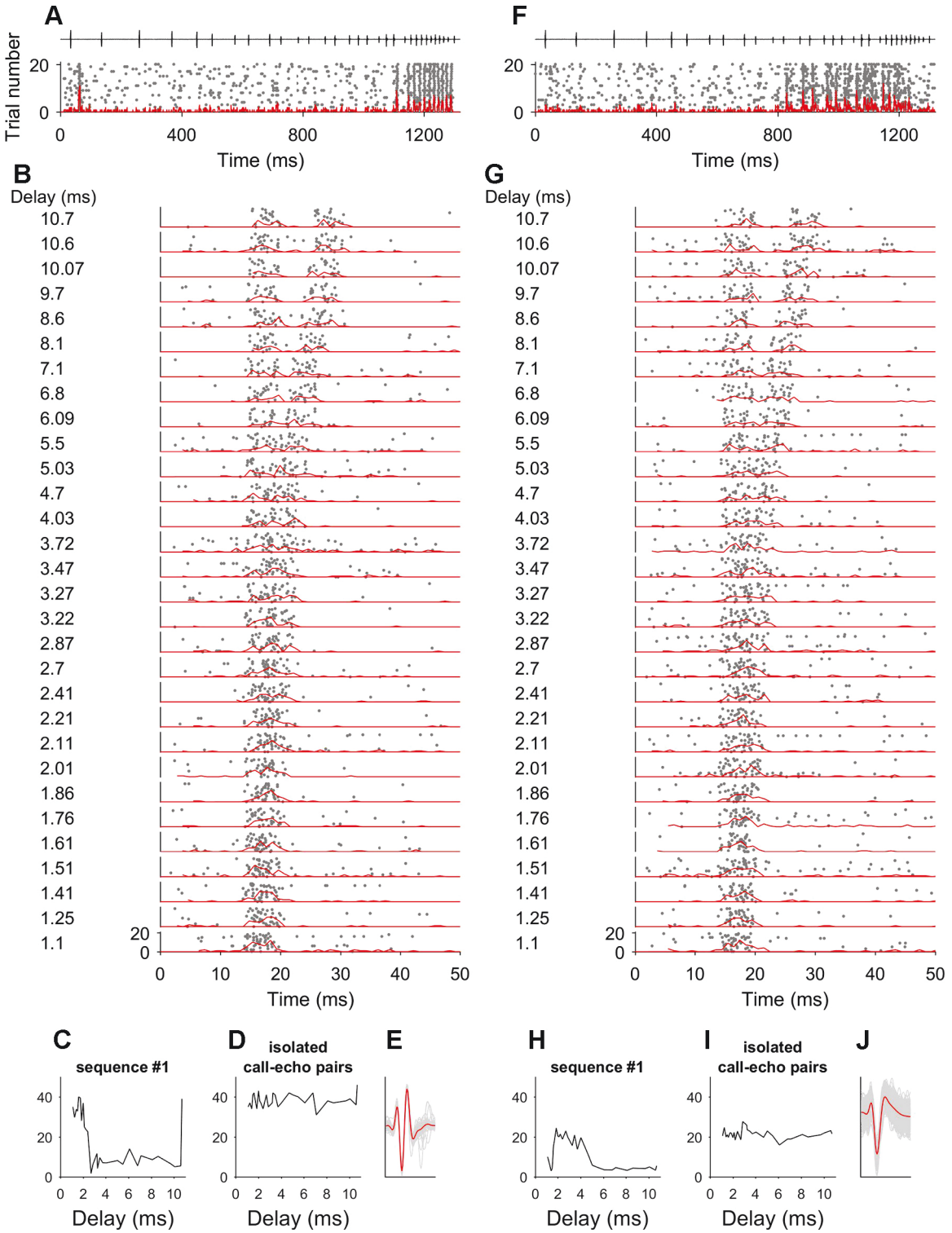

Fig. 8. Responses of two selective inferior colliculus (IC) neurons to *sequence 1* and isolated call-echo pairs. *A*: *top*, waveform of *sequence 1*; *bottom*, dot raster display (gray dots) and poststimulus time histograms (PSTH, red line, 1-ms bin) of the response. *B*: *left*, delay of each isolated call-echo pair; *right*, response to each of the isolated call-echo pairs. *C*: delay tuning curve calculated to the response in *A*; selectivity index = 0.66. *D*: delay tuning curve calculated for the response in *B*; selectivity index = 0. *E*: 2-ms segment of overlapping action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting. *F*: response to a different unit to *sequence 1*. *G*: responses to the isolated call-echo pairs. *H*: delay tuning curve calculated for the response in *E*; selectivity index = 0.63. *I*: delay tuning curve calculated for the response in *B*; selectivity index = 0. *J*: 2-ms segment of overlapping action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting.

natural sequence was significantly greater than the response selectivity to the isolated call-echo pair condition (0.76 ± 0.12 vs. 0.28 ± 0.17; Wilcoxon signed-rank test, $P < 0.001$).

Response Selectivity to Element-Shuffled and Time-Reversed Echolocation Sequences

To determine if pulse interval specifically drove response selectivity, we studied 56 neurons with a stimulus sequence in which the order of each call-echo pair was shuffled randomly, but still preserved the call-echo delays of natural stimulus sequences. The response of a neuron to *sequence 1* and the shuffled sequence is presented in Fig. 9. When stimulated with *sequence 1*, this neuron showed a selective response to call-echo delays between 1.1 and 2.1 ms (Fig. 9, *A* and *B*). The use of a shuffled sequence as stimulus provided us with the opportunity to separate echo-delay tuning from selectivity to pulse interval. If neurons were tuned to pulse interval then the response pulse interval functions to the natural sequence and the shuffled sequence should be similar, and the response echo-delay function should be different. If neurons were delay tuned, then the response echo-delay function to the natural and shuffled sequences should be similar, and the response pulse interval function should differ. In the unit shown in Fig. 9, as well as in the remaining 55 units tested with natural and shuffled sequence stimuli, the number of spikes in the echo-delay function were similar (compare Fig. 9, *B* and *E*), and the number of spikes in the pulse interval function were different (compare Fig. 9, *C* and *F*). There were no significant differences between best delays measured in response to the natural sequence and in response to the shuffled sequence (Wilcoxon signed-rank test, $P = 0.77$; Fig. 9*G*).

To determine if neurons responded to specific call-echo delays or to a specific stimulus order in the sequence, we studied 56 units with a time-reversed natural sequence such that call-echo elements with shortest delays were presented at the start of each sequence, and longest delays were presented at the end. In this condition, the individual call-echo elements were preserved in their original form such that the call still preceded the echo and both were downward frequency modulated. An example neuron responding to *sequence 1* and the reversed sequence is shown in Fig. 10. This unit responded selectively to call-echo delays between 6.09 and 8.6 ms when stimulated with the natural sequence (Fig. 10, *A* and *B*) and to call-echo delays between 6.8 and 9.7 ms when stimulated with the reversed sequence (Fig. 10, *C* and *D*). Among the 56 units included in this analysis, there were no significant differences between the best delays measured with the natural sequence and the time-reversed sequence (Wilcoxon signed-rank test, $P = 0.22$; Fig. 10*E*). Thus, the IC neurons sampled here showed selectivity to call-echo delay and not stimulus order in the sequence.

Pulse Interval Shapes Echo-Delay Response Selectivity

The results from the shuffled sequence and time-reversed sequences showed that the echo-delay tuning is not determined by the history of pulse interval in natural sonar sequences.

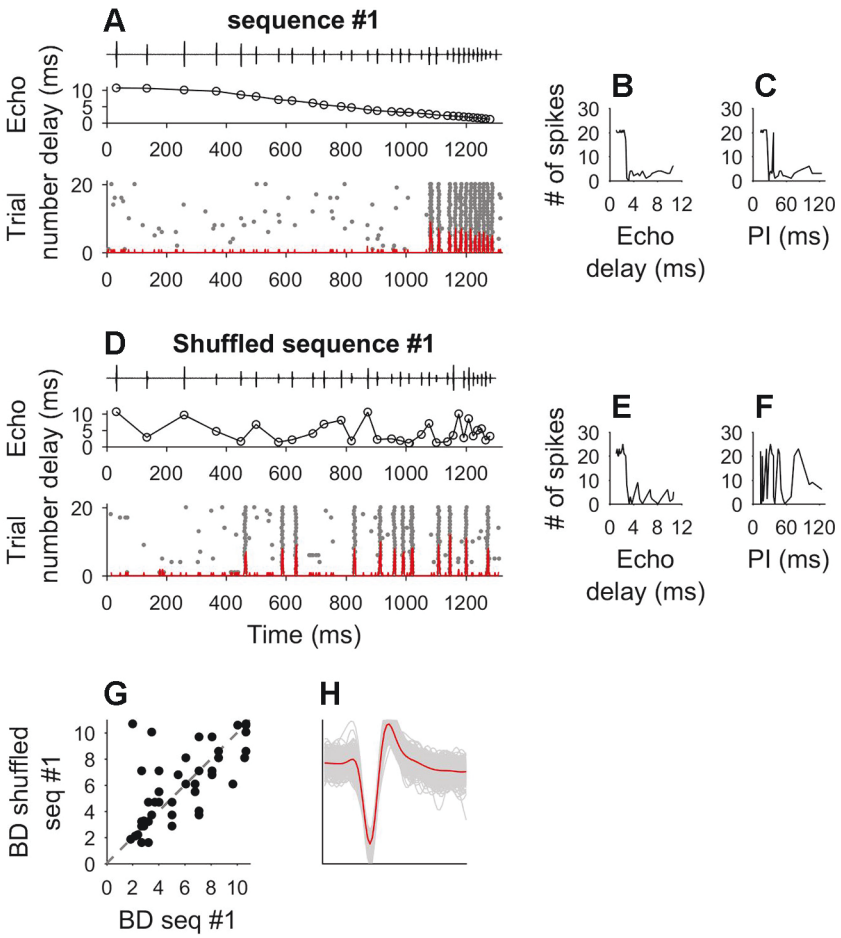

Fig. 9. Responses of one selective inferior colliculus (IC) neuron to the shuffled *sequence 1*. *A*: *top*, waveform of *sequence 1*; *middle*, variation of call-echo delay across time in *sequence 1*; *bottom*, dot raster display and poststimulus time histograms (PSTH) of the response to *sequence 1*. *B*: delay tuning curve calculated from the response in *A*. *C*: no. of spikes as a function of the pulse interval (PI) for the response in *A*. *D*: *top*, waveform of the shuffled *sequence 1*; *middle*, variation of call-echo delay across time in shuffled *sequence 1*; *bottom*, dot raster display and PSTH of the response to shuffled *sequence 1*. *E*: delay tuning curve calculated from the response in *D*. *F*: no. of spikes as a function of the pulse interval for the response in *D*. *G*: comparison between best delay measured in *sequence 1* and best delay (BD) measured in the shuffled sequence (Wilcoxon signed-rank test, $P = 0.77$, $n = 56$ units). *H*: 2-ms segment of overlapping action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting of the unit shown in *A*–*F*.

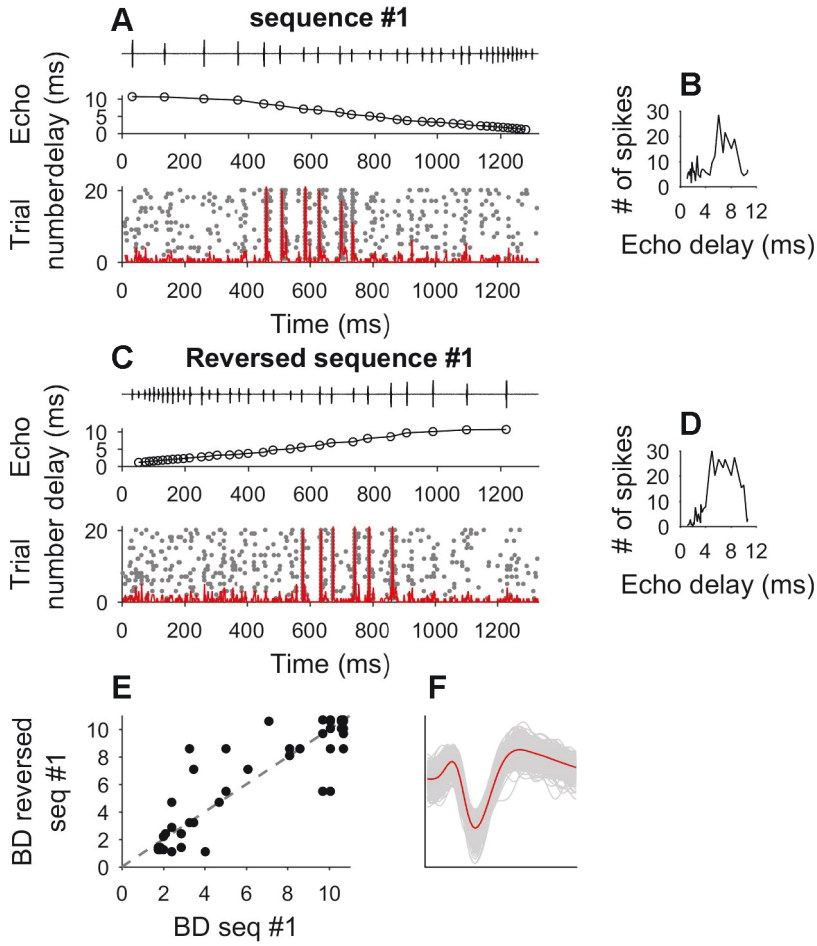

Fig. 10. Response of one selective inferior colliculus (IC) neuron to the reversed *sequence 1*. *A*: *top*, waveform of *sequence 1*; *middle*, variation of call-echo delay across time in *sequence 1*; *bottom*, dot raster display and poststimulus time histograms (PSTH) of the response to *sequence 1*. *B*: delay tuning curve calculated from the response in *A*. *C*: *top*, waveform of the shuffled *sequence 1*; *middle*, variation of call-echo delay across time in reversed *sequence 1*; *bottom*, dot raster display and PSTH of the response to reversed *sequence 1*. *E*: delay tuning curve calculated from the response in *C*. Comparison between best delay measured in *sequence 1* and best delay measured in the reversed sequence (Wilcoxon signed-rank test, $P = 0.22$, $n = 56$ units). BD, best delay. *F*: 2-ms segment of overlapping action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting of the unit shown in *A–D*.

Combining this with the result showing a lack of the neural selectivity to the isolated call-echo pairs presented at a 300-ms interstimulus interval, we hypothesized that the interstimulus interval of the natural sequence is the crucial parameter affecting the neural selectivity. To directly test this hypothesis, we studied 54 units using two additional sequences with fixed PI of either 50 or 250 ms. Both sequences maintained the same order of call-echo presentation and time-frequency structure of elements in the natural sequence. Figure 11 shows the responses of one neuron to the natural (Fig. 11*A*), the 50-ms PI (Fig. 11*B*), and the 250-ms PI (Fig. 11*C*) sequences. This unit responded selectively to call-echo delays between 3.47 and 6.8 ms when stimulated with the natural sequence (Fig. 11*D*) and to delays between 4.03 and 6.8 ms when stimulated with the 50-ms PI sequence (Fig. 11*E*). However, this neuron showed no selectivity in its response to call-echo delay when stimulated with the 250-ms PI sequence (Fig. 11*F*). In addition, the responses to the individual call-echo elements in the 250-ms PI sequence were similar to those to the isolated call-echo elements (Fig. 11*G*). Overall, this pattern of results was observed in the 54 units tested. The selectivity indexes calculated for the natural sequence and the 50-ms PI sequence were not significantly different, but both were significantly greater than the selectivity index to the 250-ms PI sequence and for the randomly presented isolated call-echo pairs (repeated-measures ANOVA, $P < 0.05$, Fig. 11*H*).

Selective Responses Depend on the Acoustic Spectrotemporal Features of Natural Stimulus Elements

To investigate the effect of the spectrotemporal features of natural calls and echoes on the delay-tuned neural responses, artificial echolocation sequences were generated in which one or more acoustic parameters, such as the bandwidth or the duration of the call-echo pair, was manipulated in artificial sequences, which contained the same number of stimulus elements as the natural sequences. First, we compared the response of 31 neurons with natural *sequence 1* and with an artificial sequence, consisting of one-harmonic logarithmic sweeps with constant 80-kHz bandwidth of calls and echoes (between 100 and 20 kHz), while all other parameters (temporal and intensity) were matched to the natural echolocation *sequence 1*. We refer to this sequence as Artificial FM Natural Duration. An example response is shown in Fig. 12. This neuron showed a selective response to delays between 1.1 and 2.4 ms when stimulated with the natural sequence (Fig. 12, *A* and *B*) and a similar response pattern to the artificial FM sequence (Fig. 12, *C* and *D*). This pattern was observed in 21 neurons (Fig. 12*E*) in which the best delays were similar between the natural and the artificial stimuli (Wilcoxon signed-rank test, $P = 0.90$). However, the strength of the response, quantified as the spike rate at the BD at each of the call-echo pairs, was consistently lower to the artificial sequence than the natural sequence (Fig. 12*F*, Wilcoxon signed-rank test, $P < 0.001$). Furthermore, in 10 of the 31 neurons, the artificial FM

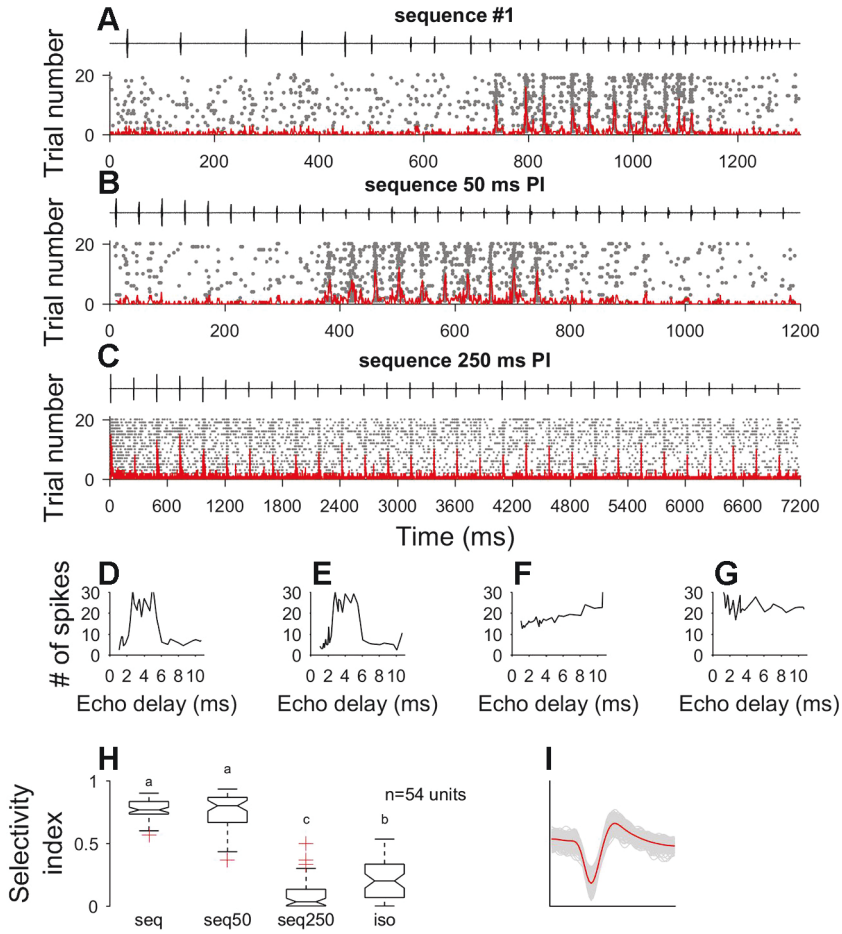

Fig. 11. Response of one inferior colliculus (IC) neuron to the natural and pulse interval manipulated echolocation sequences. *A*: response to natural echolocation *sequence 1*. *B*: response of the neuron shown in *A* to a manipulated *sequence 1* in which the order of appearance of the call-echo pairs is maintained, but the pulse interval is kept constant at 50 ms. *C*: response of the neuron shown in *A* to a manipulated *sequence 1* in which the order of appearance of the call-echo pairs is maintained but the pulse interval is kept constant at 250 ms. *D*: delay tuning curve obtained with the natural sequence. *E*: delay tuning curve obtained with manipulated sequence of 50-ms pulse interval (PI). *F*: delay tuning curve obtained with manipulated sequence of 250 ms PI. *G*: delay tuning curve obtained with the randomly presented individual call-echo pairs at 300-ms intervals. *H*: comparison of the selectivity index calculated in the response to the natural sequence and the manipulated sequence (seq, natural sequence; seq50, manipulated sequence of 50 ms PI; seq250, manipulated sequence of 250 ms PI; iso, isolated call-echo pairs) for 54 units. Box-whisker plots show the median (50th percentile) as a solid red line inside the box delimited by the 25th and 75th percentiles with whiskers extending to the 10th and 90th percentile. Identical letters indicate no statistical differences (repeated-measures ANOVA, $P < 0.05$, $n = 54$ units). *I*: 2-ms segment of overlapping action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting of the unit shown in *A*–*G*.

sequence did not evoke a selective response. An example is depicted in Fig. 13. This unit showed an echo delay tuned response between 5 and 8 ms (Fig. 13, *A* and *B*) to the natural sequence, which included changes in the spectrotemporal structure of stimulus elements, whereas this unit showed no response to the artificial sequence (Fig. 13, *C* and *D*). This indicates that ~1/3 of the sampled neurons are sensitive to the covariation of spectrotemporal parameters in the natural sequence.

Second, we compared the response of 31 units between the natural *sequence 1* and two other artificial sequences as follows: *1*) one artificial sequence, consisting of one-harmonic logarithmic sweeps with constant 80-kHz bandwidth of calls and echoes and a fixed duration of 3 ms (Artificial FM 3 ms) and *2*) one artificial sequence with similar constant bandwidth of calls and echoes and a fixed duration of 1 ms (Artificial FM 1 ms). The response of one unit to these sequences is shown in Fig. 14. This neuron showed a selective response to call-echo delays between 2.8 and 6 ms when stimulated with the natural sequence (Fig. 14, *A* and *B*). In response to the artificial FM sequences, this neuron showed an increased response to a similar range of delays, between 2.8 and 6 ms, compared with the natural sequence (Fig. 14, *C*–*F*). The same pattern was observed in 21 neurons. Best delays measured in response to the natural sequence were similar to those measured in response to the artificial duration sequences (Fig. 14, *G* and *H*). This indicates that, in these 21 units, the call and echo duration did not influence the delay tuning profile. However, the strength of the response, quantified as the spike rate at each of the call-echo pairs, was lower for the artificial sequences compared with the natural sequence (Fig. 14*I*, Kruskal-Wallis test, $P < 0.001$).

DISCUSSION

Here, we report new evidence that the midbrain IC of the awake FM bat, *E. fuscus*, contains a population of neurons that show response selectivity to the time delay between call-echo pairs. Our main findings are: *1*) call-echo delay selectivity is present in the IC of the FM bat, *E. fuscus*, when neurons are stimulated with call-echo pairs presented in sequences that capture the temporal dynamics of the bat's natural sonar behavior; and *2*) pulse interval within echolocation sequences and the natural spectrotemporal fine structure of sonar signals can modulate neuronal selectivity to call-echo delay. Our findings have implications for understanding general mechanisms of sound processing in species that must analyze temporal acoustic patterns to execute natural behaviors.

Some IC neurons may acquire response selectivity from corticofugal projections (Huffman and Henson 1990). In bat auditory cortex, the receptive fields of echo-delay tuned neurons exhibit response selectivity that depends on combinations of acoustic stimulus parameters. For example, best echo-delay of cortical neurons changes with pulse and echo amplitude and with signal duration (Dear et al. 1993; Hagemann et al. 2010, 2011; Macías et al. 2016; Sullivan 1982; Tanaka et al. 1992).

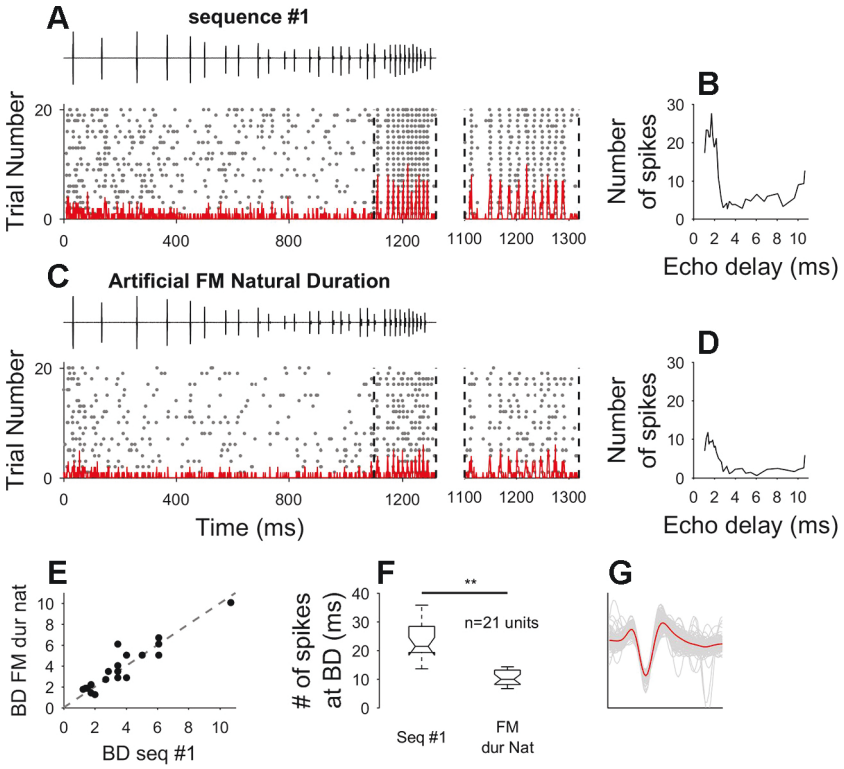

Fig. 12. Responses of one inferior colliculus (IC) neuron to *sequence 1* and artificial frequency modulated (FM) sweeps sequence "Artificial FM Natural Duration." *A*: example of a unit responding to the natural echolocation *sequence 1* [*top*, oscillogram of the pulse only sequence; *bottom right*, dot raster display (gray dots) and poststimulus time histograms (PSTH, red line, 1-ms bin) of the response; *bottom left*, detailed raster and PSTH of the response]. *B*: delay tuning curve calculated for this response. *C*: response of the same unit to an artificial FM sweeps sequence where the bandwidth and duration of each element matched those of the natural sequence (Artificial FM Natural Duration). *D*: delay tuning curve of the response to Artificial FM Natural Duration. *E*: comparison of the best delays (BD) measured at the natural sequence and the Artificial FM Natural Duration sequence in 21 units (Wilcoxon signed-rank test, $P = 0.90$). *F*: comparison of the no. of spikes measured at the best delay in the response with the natural sequence and the artificial FM sweeps sequences for 21 units (Wilcoxon signed-rank test, $^{**}P < 0.001$). Box-whisker plots show the median (50th percentile) as a solid red line inside the box delimited by the 25th and 75th percentiles with whiskers extending to the 10th and 90th percentile. *G*: 2-ms segment of overlapping action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting of the unit shown in *A–D*.

In a subpopulation of neurons in the auditory cortex of the mustached bat, best delay shortens and tuning sharpens as the repetition rate of pulse-echo pair stimuli increases (O'Neill and Suga 1982).

Studies employing naturalistic echolocation stimuli have revealed additional properties of echo-delay tuned neurons in the auditory cortex of FM bat species. For example, auditory cortical neurons of the big brown bat, *E. fuscus*, show echo-delay tuned responses to simulated sonar returns from two closely spaced reflecting surfaces, giving rise to spectral interference patterns (Sanderson and Simmons 2002). Auditory cortical neurons in the short-tailed fruit bat, *C. perspicillata*, show sharper echo-delay tuning when stimulated with natural echolocation sequences compared with temporally isolated call-echo pairs (Beetz et al. 2016). The cortical chronotopic map of echo delay in the FM bat *Phyllostomus discolor* is modified by dynamic acoustic streams that simulate echo flow patterns the bat may receive as it flies, and the cortical representation of close-range targets is enlarged when the simulated lateral passing distance of objects decreases (Bartenstein et al.

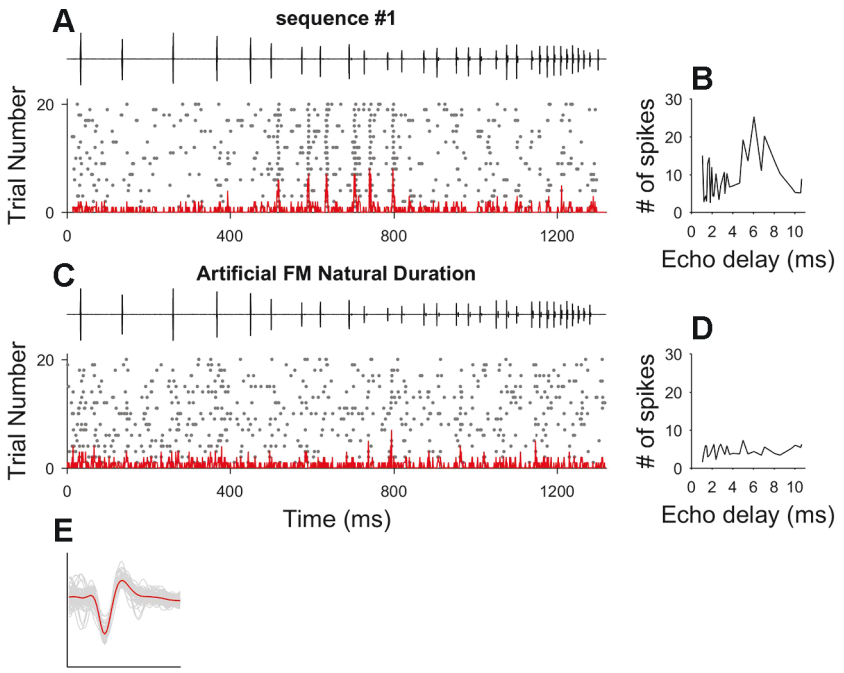

Fig. 13. Neuronal responses to the natural sequence and artificial frequency modulated (FM) sweeps sequence "Artificial FM Natural Duration." *A*: example of a unit responding to natural echolocation *sequence 1*. *B*: delay tuning curve calculated for this response. *C*: response of the same unit to an artificial FM sweeps sequence where the bandwidth and duration of each element matched those of the natural sequence (Artificial FM Natural Duration). *D*: delay tuning curve of the response to Artificial FM Natural Duration. *E*: 2-ms segment of overlapping action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting.

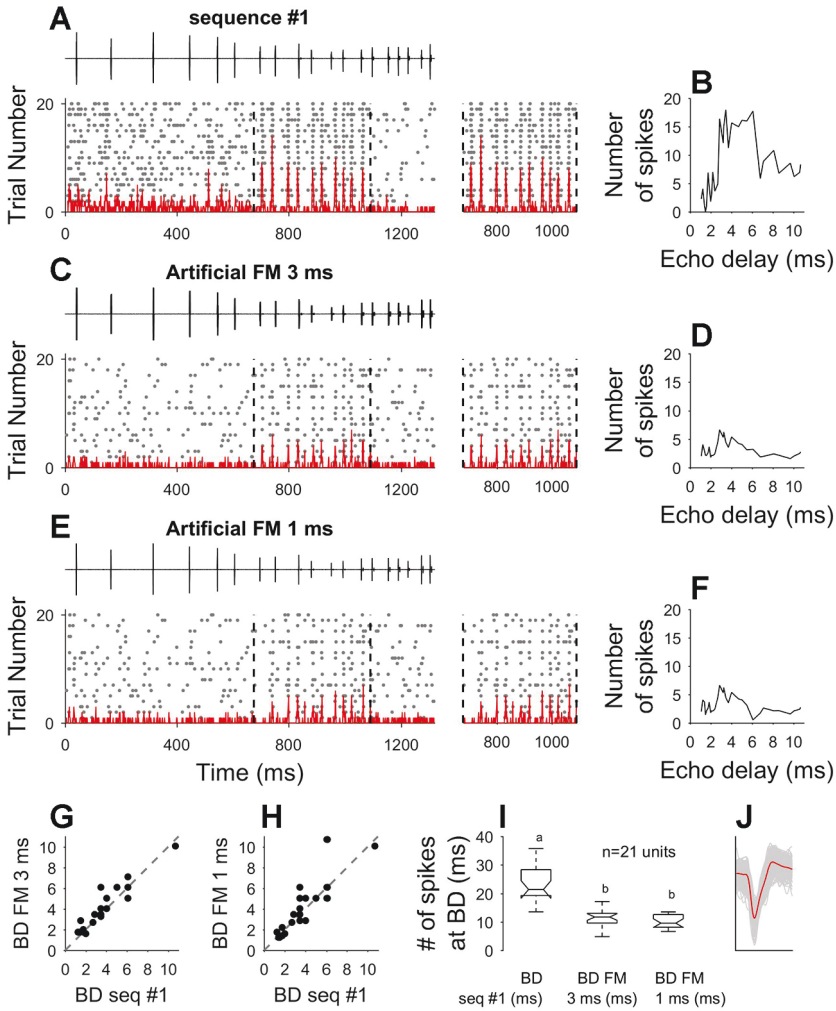

Fig. 14. Neural responses to the natural sequence and artificial frequency modulated (FM) sweeps sequence "Artificial FM 3 ms" and "Artificial FM 1 ms." *A*: example of a single inferior colliculus (IC) neuron responding to the natural echolocation *sequence 1* [*top*, oscillogram of the pulse only sequence; *bottom right*, dot raster display (gray dots) and poststimulus time histograms (PSTH, red line, 1-ms bin) of the response; *bottom left*, detailed raster and PSTH of the response]. *B*: delay tuning curve calculated for this response. *C*: response of the same neuron to an artificial FM sweep sequence where the bandwidth and duration of pulses and echoes were kept constant. FM duration was 3 ms (Artificial FM 3 ms). *D*: delay tuning curve of the response to Artificial FM 3 ms. *E*: response of the same unit to an artificial FM sweep sequence where the bandwidth and duration of pulses and echoes were kept constant. FM duration was 1 ms (Artificial FM 1 ms). *F*: delay tuning curve of the response to FM 1 ms. *G*: comparison of the best delays (BD) measured at the natural sequence and the FM 3-ms sequence (Wilcoxon signed-rank test, $P = 0.7$). *H*: comparison of the BD measured at the natural sequence and the FM 1-ms sequence (Wilcoxon signed-rank test, $P = 0.27$). *I*: comparison of the no. of spikes measured at the best delay in the response to the natural sequence and the artificial FM sweeps sequences FM 3 ms and FM 1 ms, for 21 units (Kruskal-Wallis test). Box-whisker plots show the median (50th percentile) as a solid red line inside the box delimited by the 25th and 75th percentiles with whiskers extending to the 10th and 90th percentile. Identical letters indicate no statistical differences. *J*: 2-ms segment of overlapping action potential waveforms (*y*-axis in arbitrary units) obtained after spike sorting shown in *A*–*F*.

2014). Dynamic cortical activity patterns in bats may be mediated by midbrain selectivity to natural acoustic stimulus parameters.

Sanderson and Simmons (2005) investigated midbrain IC responses in *E. fuscus* to natural echolocation sequences and reported neural selectivity to stimulus elements within natural call-echo sequences. This study did not directly investigate neuronal echo-delay selectivity, but discovered that *1*) echolocation pulse repetition rate serves to sharpen the resolution of the sonar receiver and *2*) the latency of population responses to natural sequences can be used to encode target distance.

In the midbrain IC of the CF-FM bat, *Pteronotus parnellii*, populations of delay-tuned eurons respond selectively to combinations of simulated sonar calls and echoes [or tones falling within the frequency-modulated component of the 1st and 2nd–4th harmonics in echolocation calls of the mustached bat (*P. parnellii*)] but only weakly or not at all to calls or echoes alone (Portfors and Wenstrup 1999; Yan and Suga 1996). By contrast, earlier studies of the IC in insectivorous bat species that use only FM signals failed to identify neurons showing selectivity to call-echo delay (Feng 2011; Feng et al. 1978). Our findings suggest that species differences in temporal processing are driven by differential sensitivity to stimulus context: echo delay tuning of neurons in the IC of the insectivorous CF-FM bat, *P. parnelli*, appears in response to temporally isolated simulated call-echo pairs, whereas echo-delay response selectivity in the FM bat, *E. fuscus*, emerges in the IC only when acoustic stimuli are presented at rates of natural insect sonar pursuit sequences. In addition, some IC responses in *E. fuscus* depend on the combination of the natural spectrotemporal structure of echolocation signals and their temporal patterning within a dynamic sequence. Species differences in neural selectivity to echo-delay may have coevolved with specializations in sonar signal design in insectivorous bats.

Neural response properties used to characterize echo-delay tuning include the strength of facilitation and distribution of best delays. The strength of facilitation provides a measure of the magnitude of a neuron's response to the combination of a simulated call and echo compared with its responses to each of the stimulus components separately (Olsen and Suga 1991; Portfors and Wenstrup 1999; Valentine and Moss 1997). Most of the echo-delay tuned neurons we studied in *E. fuscus* showed little or no response to the presentation of the call or the echo alone. The average response of IC neurons to the echo was 0.2 ± 0.02 spikes/stimulus, and the average response to the call was 0.3 ± 0.02 spikes/stimulus. However, when both call and echo were combined at the appropriate delay, the response was facilitated, with an average response of 1.3 ± 0.6 spikes/stimulus, which is similar to that reported for the IC of

the CF-FM mustached bat, *P. parnellii* (Portfors and Wenstrup 1999).

We found that the facilitated responses to specific echo delays of IC neurons are locked to the echo arrival time. This indicates that IC neurons in the FM bat, *E. fuscus*, respond to restricted time intervals between calls and echoes, which is the defining characteristic of echo-delay tuned neurons (Dear and Suga 1995; Hechavarría et al. 2013b; O'Neill and Suga 1982; Suga and O'Neill 1979; Sullivan 1982; Valentine and Moss 1997). The best delays in our sample of IC neurons in *E. fuscus* ranged between 1.1 and 10.7 ms, which includes responses to echo delays present in the natural biosonar sequence. The stimuli in our study came from acoustic recordings of bats tracking a target at a maximum distance of ~2 meters, returning echoes at maximum delays of ~12 ms, and therefore precluded presentation of longer echo delays.

Our data show an overrepresentation of short best delays around 2 ms (73% are $<$4 ms). This is in contrast to the best delay distributions reported in the superior colliculus (Valentine and Moss 1997) and intertectal nucleus (Dear and Suga 1995) of *E. fuscus*, which show a predominance of longer best delays. In the auditory cortex of *E. fuscus*, best delays below 3 ms are also not prominent (Dear et al. 1993).

One possible explanation for discrepant reports on the distribution of neural best echo-delays in the bat auditory pathway could be differences in the repetition rates used for acoustic stimulation across studies. Increasing the rate of stimulus presentation can drive cortical delay-tuned neurons to decrease their best delay (O'Neill and Suga 1982). In our study, echo-delay tuned responses occurred largely for pulse intervals in the natural sequence, which ranged between 14 and 124 ms. Therefore, neurons tuned to short delays were stimulated at shorter pulse intervals (between 14 and 40 ms PI), which is lower than stimulus intervals presented by Valentine and Moss (1997), 200 ms; Dear and Suga (1995), 100 ms; and Dear et al. (1993), 200 ms. It is therefore possible that the high stimulus presentation rate at the final stages of the natural echolocation sequences could have driven the short echo-delay tuned responses reported here.

Another potential explanation for the overrepresentation of neurons tuned to short echo-delays in our study could be the call/echo levels in the natural echolocation sequence. In the AC of *P. parnellii*, *C. perspicillata*, and *P. quadridens*, delay-tuned neurons show tilted receptive fields, that is, increasing echo level drives neurons to respond to shorter echo delays (Hechavarría et al. 2013b). In the natural sonar *sequence 1* used in this study, echo level increased from 28 to 54 dB SPL with decreasing target distance, which might have driven neurons to respond to shorter delays.

IC response latencies below 8–10 ms are too short to derive echo-delay tuning characteristics from cortical inputs (Dear et al. 1993; Hechavarría et al. 2013b; O'Neill 1995), indicating that some IC neurons derive these echo-delay tuning properties from brain stem and/or IC circuitry. Our data show echo response latencies in some IC neurons that fall below 5 ms, which is consistent with earlier findings. For example, Haplea et al. (1994) reported latencies of IC neurons in *E. fuscus* as short as 2.9 ms. In the IC of the mustached bat, *P. parnellii*, neural response latencies to high-frequency sound stimuli, which mimic echoes in call-echo pair combinations, occur below 10 ms, with some units responding 5 ms after the echo (Portfors and Wenstrup 1999).

Freely behaving *E. fuscus* decrease pulse interval and produce sonar sound groups when they approach and inspect objects (Kothari et al. 2014; Moss et al. 2006; Petrites et al. 2009; Sändig et al. 2014). Adaptive adjustments in pulse interval not only increase the number of echoes a bat receives per unit time but also influence neuronal selectivity to target range or echo delay. Our study of midbrain IC neurons in the big brown bat revealed a population with echo-delay tuned responses to stimuli presented at pulse intervals shorter than 124 ms (longest pulse interval within the natural sonar sequence). At pulse intervals of 250 ms, these same neurons did not show echo-delay tuning. In extracellular recordings from the midbrain SC of free-flying *E. fuscus*, echoes from physical objects evoked delay-tuned responses in single neurons, and echo-delay tuning sharpened and shifted to shorter delays when the bat produced sonar sound groups (Kothari et al. 2018). This shows that target range representation in the midbrain can be modulated by the bat's active control over call timing.

Acoustic stimulation with temporally patterned pulse trains at varied sound repetition rates can also affect neural response selectivity to combinations of sound amplitude, direction, duration, and frequency (Casseday et al. 1994; Casseday and Covey 1995; Jen and Zhou 1999; Jen et al. 2001; Klug et al. 1995; Koch and Grothe 1998; Le Beau et al. 1996; Wu and Jen 1996; Zhou and Jen 2001). Our results, however, suggest that the history of pulse interval does not specifically determine echo-delay tuning of IC neurons in *E. fuscus*, since stimulus selectivity does not change between call-echo delay shuffled and natural sonar sequences, if the pulse interval is within the range occurring in natural sequences.

Our data showing echo-delay tuning in a population of IC neurons in the awake insectivorous bat, *E. fuscus*, differ from recently published data on IC neurons in the anesthetized frugivorous bat, *C. perspicillata* (Beetz et al. 2017). IC neurons in *C. perspicillata* responded to all acoustic elements within an echolocation sequence, but with increased firing rate to a subset of the call-echo pairs in the sequence, indicating echo-delay modulation. It should be noted that frugivorous bats, such as *C. perspicillata*, use echo delay to measure distance to stationary objects, not moving prey. In addition, frugivorous bat species may use visual orientation or olfaction to find food (Thies et al. 1998). *C. perspicillata* have ultraviolet-sensitive cones, which may be advantageous for visual orientation (Müller et al. 2009). All of these factors, along with possible effects of anesthesia on neural responses in *C. perspicillata*, may account for reported differences in IC echo-delay selectivity.

The echo-delay response selectivity reported here for neurons in the IC of *E. fuscus* motivates the hypothesis that auditory activity in the FM bat is gated by the animal's adaptive control over pulse interval, which shapes neuronal selectivity to objects at different distances or echo delays (Kothari et al. 2018). Furthermore, these data show that response selectivity of IC neurons to one stimulus parameter is not context dependent [see Nelken (2004) for review].The relevance of these findings is not limited to echolocating bats but rather speaks to the general importance of stimulus context to auditory temporal processing in a wide range of species, whose survival depends on the analysis of complex patterns of sounds. Future neurophysiological research on bats and other

animals can further elucidate the neural mechanisms of complex signal processing in natural contexts.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.M., J.L., and C.F.M. conceived and designed research; S.M. and J.L. performed experiments; S.M. analyzed data; S.M., J.L., and C.F.M. interpreted results of experiments; S.M. prepared figures; S.M. and J.L. drafted manuscript; S.M., J.L., and C.F.M. edited and revised manuscript; S.M., J.L., and C.F.M. approved final version of manuscript.

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