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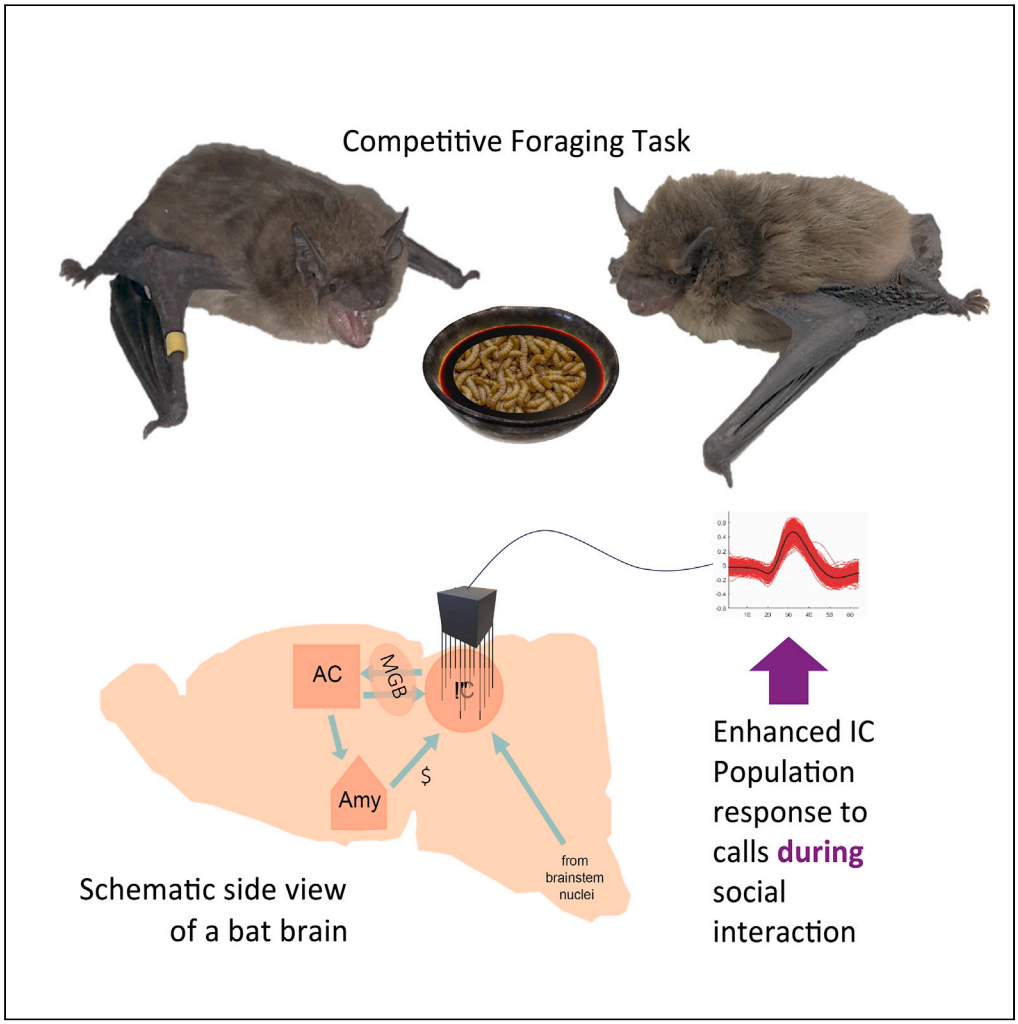

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Highlights

A competitive foraging task elicits a wide array of social behaviors in bats

IC recordings of bats revealed stronger responses to calls during social events

Neural recordings were obtained from the IC of a copulating bat

Auditory processing is modulated by social context early in the auditory pathway

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Auditory processing of communication calls in interacting bats

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SUMMARY

There is strong evidence that social context plays a role in the processing of acoustic signals. Yet, the circuits and mechanisms that govern this process are still not fully understood. The insectivorous big brown bat, *Eptesicus fuscus*, emits a wide array of communication calls, including food-claiming calls, aggressive calls, and appeasement calls. We implemented a competitive foraging task to explore the influence of behavioral context on auditory midbrain responses to conspecific social calls. We recorded neural population responses from the inferior colliculus (IC) of freely interacting bats and analyzed data with respect to social context. Analysis of our neural recordings from the IC shows stronger population responses to individual calls during social events. For the first time, neural recordings from the IC of a copulating bat were obtained. Our results indicate that social context enhances neuronal population responses to social vocalizations in the bat IC.

INTRODUCTION

Bats are auditory specialists that have evolved to accurately discriminate differences in the acoustic features of echoes reflecting from objects in their environment. This echolocation system enables them to represent differences in the timing and spectral content of the auditory signals.[1–5] As social animals, bats also exploit a precise sound production and processing system for acoustic communication.

There are over 1,400 bat species that live in roosts ranging in size from a few dozen to millions of individuals. As such, they communicate with conspecifics to resolve territorial conflicts, interact with mates, and elicit parental care and other behavioral interactions. Big brown bats produce a wide repertoire of acoustic communication signals that vary in duration, bandwidth, and spectral profile.[6–8] Most of these calls have only been broadly characterized as either appeasement, aggression, or isolation calls, but specific behavioral functions of most calls are not fully characterized. One striking exception is the frequency-modulated bout (FMB) that big brown bats produce when engaged in competitive foraging. These calls deter conspecifics from targeting a prey item and as such, predict foraging success for the emitter.[9] The big brown bat's rich repertoire of communication calls and complex social behaviors make them ideal model animals to study the underpinnings of communication sound processing and its modulation in various social contexts.

The inferior colliculus (IC) is a midbrain structure that plays a key role in auditory information processing. It receives afferent input from brainstem nuclei and descending projections from the auditory cortex.[10,11] In several bat species, IC neurons respond to a variety of sound stimuli, including echolocation and communication calls.[12–15] IC neurons are selective to sound frequency and are arranged tonotopically, with dorsal regions containing neurons that are tuned to lower frequencies and ventral regions containing neurons tuned to higher frequencies.[12,16–18] IC neurons also show differential responses to frequency-modulated (FM) calls, with some responding only to fast downward sweeps but not to slow or upward sweeps of the same duration and bandwidth.[19] In addition, a population of IC neurons exhibits echo delay-tuning, facilitated, and selective responses to simulated call-echo pairs.[15,20,21] Some IC neurons also exhibit sound duration tuning.[22–24] Such neural response selectivity could play a role in the processing of communication calls in bat IC neurons.

Bat communication calls are commonly produced at lower sound frequencies than echolocation signals, so frequency-tuned neurons could lay the foundation for the processing of functionally distinct acoustic signals in the IC. Some communication calls, however, largely overlap the spectral content of echolocation calls but differ in fine spectrotemporal structure, which suggests that discrimination between call types also depends on the combination of many acoustic features, such as spectrum, amplitude, duration, and FM rate.

Past research shows that single neurons in the bat IC show selectivity to the features of communication calls.[14,25,26] Extensive research on the bat midbrain mechanisms of communication sound processing has been conducted on *T. brasiliensis*, which shows selectivity to the spectrotemporal patterns of social calls. This neural selectivity appears to be driven by inhibitory projections from the nucleus of the lateral

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lemniscus to the IC.[27,28] There is also evidence that selective responses of IC neurons in this bat species to communication calls are modulated by serotonin, most commonly by reducing the response strength but also, in some cases, by increasing firing rate.[29]

In the IC of *P. parnellii*, neurons respond selectively to combinations of tones at specific frequencies and delays, which have been implicated in echo ranging but may also contribute to response selectivity to species-specific communication calls.[26,30] It is noteworthy that some IC neurons respond selectively to specific social calls but may also respond to artificial combinations of pure tone frequencies contained in the social calls, suggesting that isolated elements of the social calls can drive neural activity. However, responses evoked by combinations of isolated tones are weaker than responses to natural communication calls.[31] While past work on communication sound processing in the IC of echolocating bats has generated a great deal of foundational knowledge, experiments have largely employed passive listening paradigms that omit real-time social interactions. Work in other mammals suggests conserved mechanisms for auditory processing and the potential involvement of contextual modulation at different levels in the auditory pathway (reviewed recently in[32]). We hypothesize that social encounters between conspecifics modulate auditory responses to acoustic signals, and thus, a complete understanding of auditory processing of natural sounds must employ neural recordings from freely behaving animals.

In this study, we recorded responses of IC neurons to different calls emitted by bats engaged in natural social encounters. Our experimental task engaged bats in a variety of social behaviors, and they produced a wide repertoire of communication calls. We predicted that close social interactions between individuals would boost auditory midbrain responses to social calls. The data reveal that IC responses to social calls are enhanced, evidenced by an increase in the firing rate of the recorded neurons identified by spike sorting, when the animals are actively interacting, and that the strength of the response cannot be fully explained by the sound intensity of social calls. Furthermore, we obtained neural recordings from the IC of a bat engaged in copulation and found that auditory responses to social calls were enhanced, as compared to calls produced immediately before copulation. Our findings demonstrate the role of social context on auditory processing at the level of the IC and serve as a springboard to explore the circuits involved in the modulation of auditory processing by social interactions.

RESULTS

Behavior during a competitive foraging task

Bats paired with a conspecific in a competitive foraging paradigm exhibited seven types of behavior as defined in the methods, which were documented in 120 min of video recordings over the course of 18 recording sessions, during a period of 8 months (Figure 1A, and Video S1): feeding, locomoting, echolocating, remaining still, grooming, mating, and aggressive interactions (Figure 1B). In order of prevalence, the bats spent 35.7% of the time feeding ("f") either at a dish or chewing on food retrieved from the dish; bats spent 26.2% of the time engaged in echolocation-based searching ("e"); they spent 15.4% of the time locomoting through the cage ("m"); they spent 16.7% of time remaining still ("s"); animals engaged in aggressive behaviors 3.4% of the time; bats spent (2.6%) amount of time grooming ("g"); and in one trial, we observed a mating event that lasted almost 6 min within the trial (this trial was analyzed separately, Video S2). We used DeepLabCut to annotate the distance traveled in each behavior and found that during all behaviors, except grooming, the distance traveled was significantly different from the behaviors annotated as "still" (Figure 1C). The behaviors identified as aggression events ("a") consisted of bats pushing each other with their snouts or wings. These were rapid and short bouts and usually occurred around the feeding dish when both bats approached the food. There was no statistical difference in the number of occurrences of aggressive behaviors across male-male, male-female, and female-female sex pairs (Figure 1D). Both the bat implanted for IC recordings and the non-implanted bats engaged in all behaviors equally (Figure 1E). There were no significant differences in the contribution of individual bats to the behavioral categories, with the exception of a mating event which only occurred in one trial.

Call repertoire during a competitive foraging task

During all behavioral events, bats emitted a wide repertoire of calls. We used a custom software "BattyKoda" to annotate the different types of calls following the nomenclature proposed by Gadziola and collaborators[6] and Wright and collaborators,[8] and consolidated by Montoya and colleagues[7] and identified 12 different call types emitted during the competitive foraging task (Figure 2A). The call types identified were as the following: echolocation calls, CS (chevron shaped), short frequency modulation, sHFM (shallow humped frequency modulation), DFM (downward frequency modulation), quasi-constant frequency, quasi-constant frequency – downward frequency modulation, FMB, LDFM (long downward frequency modulation), LFM (long frequency modulation), long quasi-constant frequency – chevron shaped, upward frequency modulation, and long shallow downward frequency modulation-long frequency modulation. We found that other than echolocation which constituted 71.1% of the calls emitted, the second most frequent call was the DFM call, occurring in 10.6% of the total emitted calls. Other frequently emitted calls included sHFM and FMB (Figure 2B). We identified that FMB and DFM calls appear with higher occurrence during aggression events, consistent with the reports by Gadziola and collaborators[6] and Wright and collaborators.[8] We calculated the dB SPL of the calls and found that it was highly variable across call types (Figures 2C and Table S2). FMB calls were emitted during all sex pairings, including female-female interactions, countering an earlier report from free-flying bats that only male big brown bats emit this food-claiming call (Figure 2D). FMB calls were 1.4-fold more frequent in female-female pairs than in female-male pairs and 4.2-fold more frequent in female-female pairs than in male-male pairs. There was a significantly higher number of aggression calls than appeasement calls in female-male pairings ($p < 0.05$, Mann–Whitney U test, statistic = −2.05, effect size d' = 0.79). Conversely, male-male pairings included more appeasement calls than aggression calls ($p < 0.05$, Mann–Whitney U test, statistic = 2.08, effect size d' = 1.47) (Figure 2E). In this way, the data support previous reports of behavioral context for

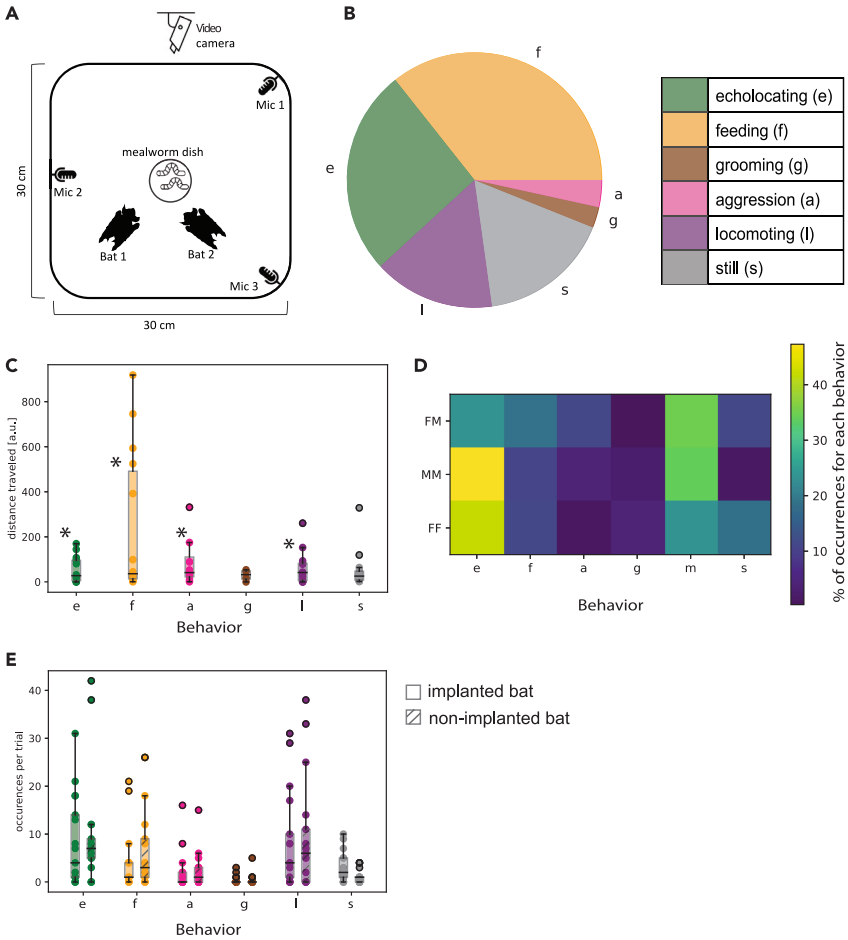

Figure 1. Bats display a wide array of behaviors during competitive foraging

(A) Experimental setup for the trained competitive foraging task.

(B) Pie chart showing the relative time spent for each defined behavior.

(C) Distance traveled across behaviors measured with DeepLabCut tracking showing significant (*$p < 0.001$ Mann–Whitney U test) movement in all behaviors except grooming when compared to the behavior defined as still.

(D) % occurrences of difference behaviors across sex pairs (each row is relative to 100%).

(E) Comparison in the occurrences per behavior across implanted and non-implanted bats showing both bats engaged in the behaviors equally.

the prevalence of different call types and show that FMB calls, previously reported to be emitted only by males, also occur between female-female pairs.

IC population responses during aggression events

We collected extracellular responses from 5 individual bats (one at a time) as each one interacted with a non-implanted bat and found that the overall evoked neuronal population response to all vocalizations was enhanced during aggression events and during grooming events (Figure 3A). When evaluating the population responses to the different types of calls, we saw that DFMs evoked significantly enhanced neuronal population activation when compared to the responses to all other calls ($p < 0.001$, Mann–Whitney U test, test statistic = 3.63, effect size d' = 0.36, Figure 3B). Yet none of the other individual call types seemed to drive enhanced neuronal firing when compared to all other call types. DFM calls are high-energy, short, downward, frequency-modulated chirps emitted by bats during aggressive encounters that usually appear as a high-repetition train of calls. These calls are broadband and fall into the human audible range. The broad bandwidth of these calls may in part explain the high number of neurons that are responsive to DFMs, as broad bandwidths may recruit neurons with different best frequencies. DFMs, as other calls, occur in several behavioral contexts and the evoked firing for each call type of behavior the animals engage in (Figure 3C) Nevertheless, we found that responses to DFM calls were highly selective (Figures 3E and 3F). Though the intensity of this type of call may in part drive the enhanced population response to DFMs, the enhancement and selectivity cannot be fully explained by the high energy of the calls, given that there is a low correlation between the dB SPL of the individual calls and the evoked spike response (Figure 3D,

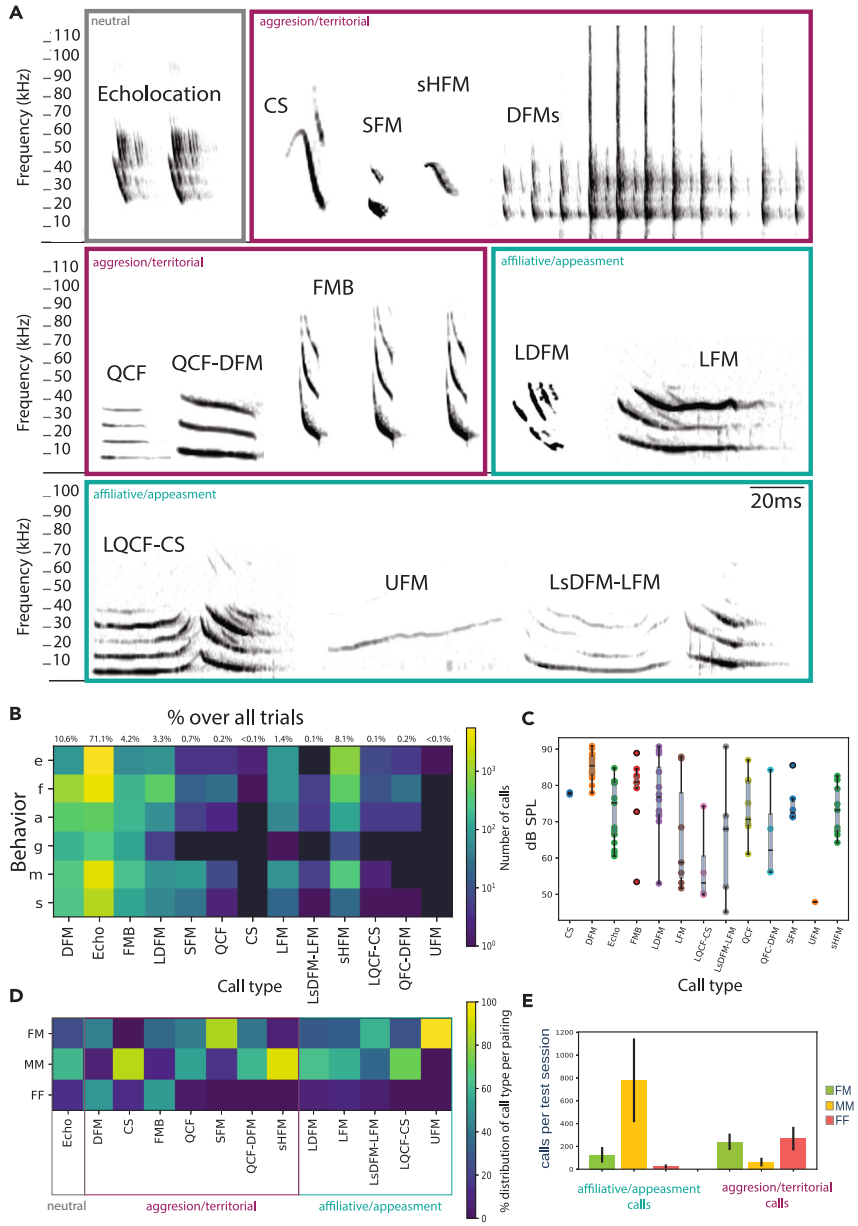

Figure 2. Social calls emitted by freely interacting bats

(A) Representative spectrograms of all types of calls recorded in this experiment, 20 ms timescale bar applies to all panels.

(B) Occurrence of calls of each type of call in each annotated behavior, top percentages represent overall occurrence across all trials.

(C) dB SPL RMS for each call type, open circles denote data points outside the quartile range.

(D) Call type occurrences across sex pairings.

(E) Average number of calls per session corresponding to each context (appeasement/affiliative vs. aggression/territoriality) per F-M (female-male), M-M (male-male), and F-F (female-female) sex pair, errorbars show SEM.

R^2 = 0.015). We did not find significant differences in call amplitude across behavioral categories (Figure S1). We evaluated the selectivity of neurons to specific calls and found that many neurons are non-selective and appear to respond to many different call types. Nevertheless, as described previously in the study by Salles et al.,[14] some neurons showed strongly selective responses to echolocation calls while some neurons showed selective responses to FMB and other social calls, such as CS and DFM (Figures 3E and 3F); neural response selectivity did not show dependence on recording depth (Figure S3). Selectivity of neurons to other call types is low. Selectivity for some calls could not be evaluated due to the lack of repetition of the calls within the trial. In this case, although some neurons showed selectivity to specific calls, the data show that social context has a strong effect on the population response of IC neurons in behaving *Eptesicus fuscus*.

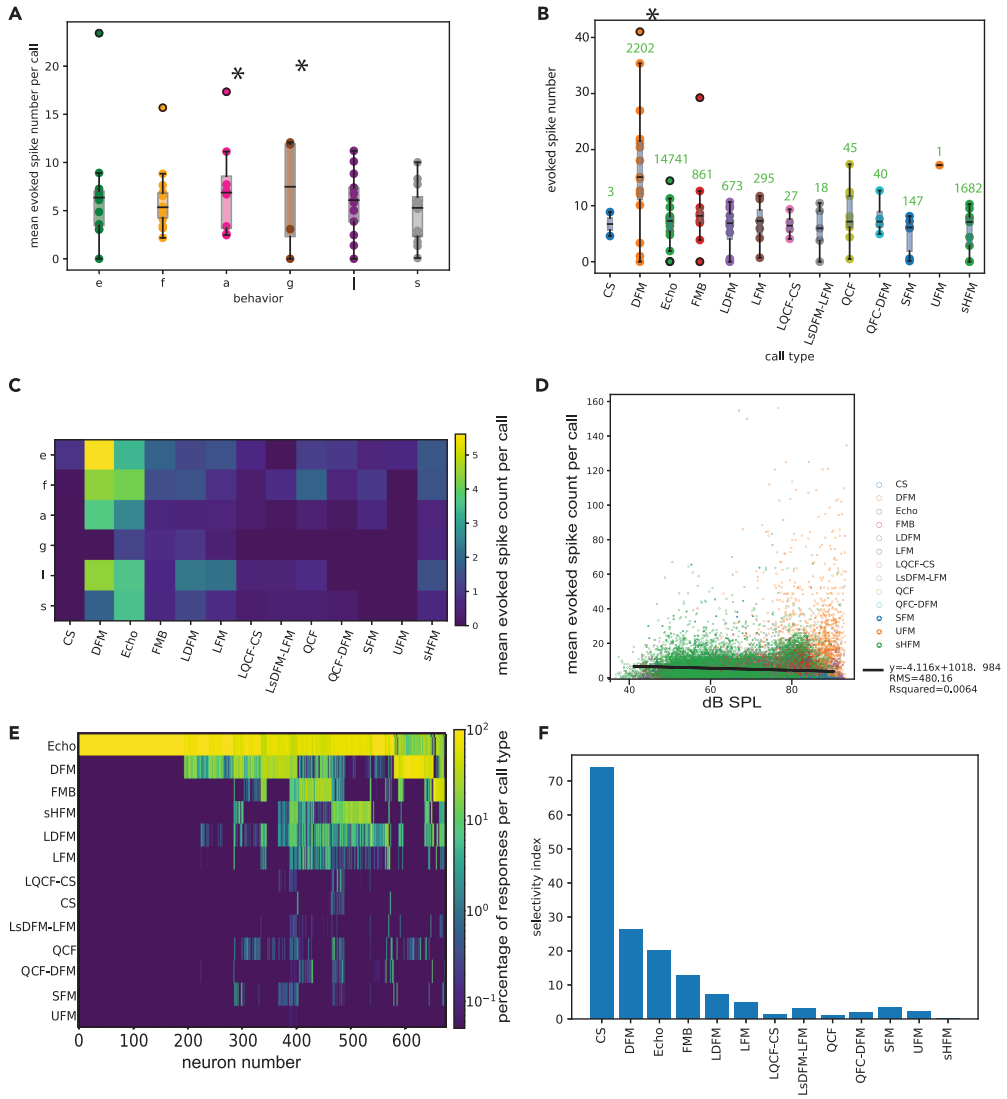

Figure 3. IC responses during social interactions

(A) Mean evoked spike count per call for each type of behavior for the neuronal population.

(B) Evoked spike number per call type, green numbers denote the number of calls included in the analysis for each call type.

(C) Population spike number per call type and per behavior.

(D) Correlation between evoked spike number and dB SPL for each call type.

(E) Percentage of responses per call type for all individual neurons.

(F) Selectivity index for units responding to a specific call calculated as the response to a specific call type over the responses of that unit to other call types.

IC population responses during mating

During one trial, a male-female bat pair engaged in mating behavior. The behavior initially appeared similar to an aggressive encounter, with the male pushing the female aside (Video S2). In this trial, the male was implanted with the neural probe. During the trial, we noticed that the LDFM call and LFM call were produced very frequently, constituting 17.4% and 9.9% of all emitted calls (Figure 4A). During all other non-mating trials, LDFM calls and LFM calls were not as frequent, occurring less than 3.3% and 1.4% over all calls, and thus we interpret that these calls are related to affiliative and/or mating behavior. We evaluated neuronal responses during mating and before the mating event and found that the neuronal population responses were significantly enhanced during mating as compared to the period before the event ($p < 0.0001$, Mann–Whitney U test, test statistic = 4.08, effect size d' = 2.50). When comparing neuronal responses to specific calls before and during mating, we see this trend across all communication call types, with differences for DFM, LDFM, and sHFM being significantly enhanced during mating (Figure 4B). Though more trials are necessary to draw strong conclusions, this event provides a window into the neural processing of natural sounds during mating in bats.

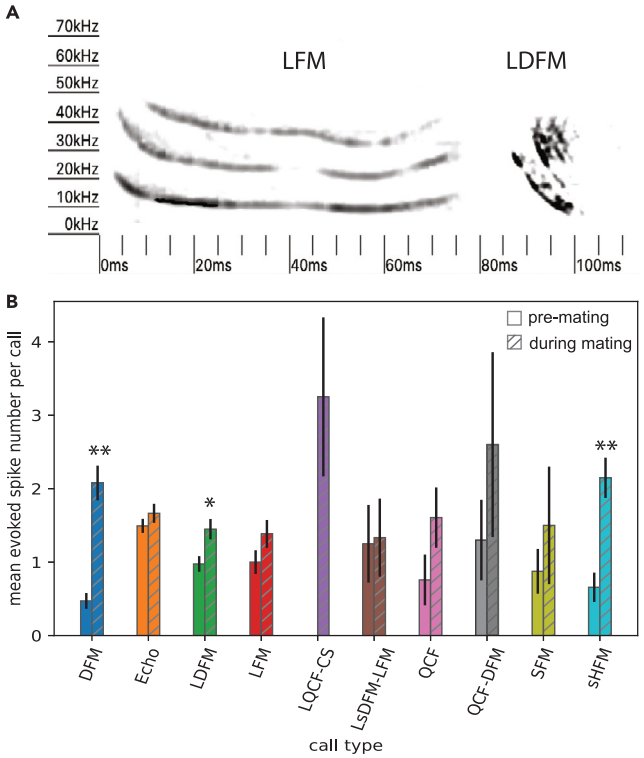

Figure 4. Mating event

(A) Spectrogram showing LFM and LDFM calls which appear overrepresented during mating behaviors.

(B) Mean evoked spike number per call type before and during mating, error bars show SEM.

DISCUSSION

The social behaviors of animals provide insights into their evolutionary adaptations and communication functions. Big brown bats (*E. fuscus*) have been studied extensively for their impressive use of echolocation for spatial orientation and navigation.[33] Nevertheless, these bats have also been the focus of research on social behaviors and vocalizations.[6–8,34] In this study, we investigated the behavior and neural responses of big brown bats during competitive foraging and one mating event. Our findings shed light on the intricate interplay between behavior and neural activity in social contexts.

When engaged in a competitive foraging task, bats exhibited a diverse range of behaviors, including feeding, locomotion, echolocation, aggression bouts, stillness, grooming, and even mating. Feeding and searching/echolocating were dominant behaviors, comprising over 60% of the observed time. Interestingly, aggression events were brief but notable, occurring primarily around feeding areas. Both male-male and female-female pairs exhibited similar patterns of aggression, indicating that sex did not significantly influence the occurrence of these behaviors. Male-male pairs exhibited more echolocation behavior, and it could be that a form of social interrogation was also taking place in this context, though we do not have sufficient data to fully evaluate this. This suggests that food competition is a primary driver of aggression in task. It is noteworthy that bats implanted with the neural recording probes and bats that were not implanted exhibited comparable frequencies of social interactions, suggesting low impact of the neural implant. During social encounters, bats emitted a variety of social calls that depended on the behavior they were engaging in, reflecting a complex communication system. Echolocation calls constituted the majority of vocalizations recorded in this experiment, consistent with their role in foraging and spatial navigation, even when crawling in the enclosure. Replicating previous findings by Gadziola and collaborators, we found specific types of syllables more frequent during aggression events and others during affiliative behavioral events.[6] Female-female pairs produced FMB calls while crawling, a finding that differs from an earlier report that these calls are produced exclusively by free-flying male big brown bats.[9] This reveals that food-claiming and territorial calls are produced by both crawling males and females of this species. However, appeasement calls varied by sex pairing, being more frequent in male-male interactions, than female-male and female-female pairings. This suggests a nuanced vocal communication strategy influenced by social dynamics.

Neural activity in this behavioral task revealed intriguing findings. Aggression events and grooming, both social behaviors, elicited enhanced neuronal population responses, indicating heightened auditory processing during social interactions that could be driven by emotional-sensory integration. Some neurons showed selectivity to specific call types that could be driven by the acoustic features of the calls. Yet, both for selective and non-selective units, spiking responses appear to have a strong dependence on behavioral context. The enhancement in neuronal responses does not appear to be primarily driven by the amplitude of the calls emitted in the behavior, yet several factors

could play a role in these responses. Notably, the DFM call elicited the most pronounced neuronal activation compared to other call types. The robust response to DFM calls, characterized by their high energy and broadband nature, suggests the influence of both call features and social context in driving neural activity. Additionally, supporting previous findings about selectivity to social calls in the IC,[14] some neurons exhibited selectivity to specific call types. For example, DFMs, FMBs, echolocation, and sHFM recruited large numbers of neurons that showed low to no responses to other calls. Nevertheless, social context appeared to have a strong influence in the modulation of neural population responses to auditory stimuli, highlighting the complex interplay between sensory stimuli and social cues in neural processing. This finding is consistent with previous research that shows contextual modulation of auditory responses in the amygdala of bats and describes direct projections from this brain region to the IC.[35,36] Furthermore, this finding is also consistent with studies in mice showing that context can modulate midbrain responses, through the corticofugal pathway.[37,38]

During a single mating event, neural responses to social calls exhibited similar patterns to other behavioral interactions. Calls associated with affiliative and mating behavior (LDFM and LFM) were significantly more frequent during a mating event than before or after, indicating their potential role in mate attraction and pair bonding. Enhanced neuronal responses of the male bat implanted with the neural probe during mating suggest heightened sensory processing, potentially reflecting the significance of vocal communication in reproductive interactions. As bats are in physical contact during mating, the intensity of calls could also be a driver for the increase in neural responses. To our knowledge, this is the first time that neural recordings have been obtained from bats engaged in mating behavior. Furthermore, we were unable to find literature describing neural recordings from the IC during mating in other species. This enhances the significance of this finding, as it provides a window into the modulation of the mammalian auditory system during mating.

This study unveils the multifaceted behaviors and neural responses of big brown bats during competitive foraging and mating events. The intricate interplay between behavior, vocalizations, and neural activity highlights the adaptive nature of social communication in these animals. The findings provide a comprehensive framework for understanding the complex social dynamics and sensory processing mechanisms in big brown bats, contributing to a broader understanding of animal behavior and communication.

Limitations of the study

A limitation of this study is that the identity of the bat that is producing the vocalizations is unknown. Unfortunately, we cannot differentiate between "own" and "other's" calls due to the closeness of the animals during interactions. With these data, reconstruction of "own" vs. "other" calls would be most challenging. We thus cannot evaluate if there are differences in the responsiveness of IC neurons to other's calls during social interaction as compared to the bat's own calls.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109872.

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AUTHOR CONTRIBUTIONS

A.S. obtained funding, designed and performed the experiments, annotated and analyzed data, and wrote the paper; E.L. helped perform experiments and annotate data; J.M. and R.M. helped annotate data; K.M.B. developed analytical tools; and C.F.M. provided scientific feedback, funding, and resources and edited the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw and analyzed data	This paper	https://github.com/angiesalles/iScience_Salles_et_al_Bats
Experimental models: Organisms/strains		
Big brown bats	Johns Hopkins University, wild caught	Species name: *Eptesicus fuscus*
Software and algorithms		
BattyKoda	Our lab	https://github.com/angiesalles/battykoda
WebKnosos	Boergens et al., 2017[39]	https://doi.org/10.1038/nmeth.4331
DeepLabCut	Mathis et al., 2018[40]	https://doi.org/10.1038/s41593-018-0209-y
JWatcher	Blumstein and Daniel, 2000[41]	https://www.jwatcher.ucla.edu/
SAS Lab Pro	Avisoft Bioacoustics	https://avisoft.com/sound-analysis/
Other		
Original python code for analysis	This paper	https://github.com/angiesalles/iScience_Salles_et_al_Bats

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Angeles Salles (salles@uic.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data is publicly available and has been deposited at https://github.com/angiesalles/iScience_Salles_et_al_Bats
- All original code is publicly available and has been deposited at https://github.com/angiesalles/iScience_Salles_et_al_Bats
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Big brown bats (*Eptesicus fuscus*) used in this study were collected from an exclusion site in Maryland under collecting permit issued by the Maryland Department of Natural Resources (no. 55440). All procedures were approved by the Institutional Animal Care and Use Committees at Johns Hopkins University (protocol no. BA17A107), where this research was conducted. Five bats were implanted with microelectrode arrays and paired with 2–5 different bats. All bats used in this study were adults, the exact age is unknown as they were wild-caught. Six female bats and 5 male bats were used for this experiment. Table S1 displays all bat pairings and sexes of the indivifuals; some bats were used as a pairing bat before serving as a neural recording subject.

METHOD DETAILS

Chronic neural implant surgery

Neural recording devices were surgically implanted in the IC of 5 bats. Bats were not fed prior to the procedure on the day of surgery but had free access to water and were fed as soon as they recovered from the anesthesia (<1 h). On the day of surgery, the bat was anesthetized with Isofluorene 4%. Then the fur over the scalp was removed with commercially available depilatory cream (Nair). The cream was applied using cotton applicators, and the fur was gently rubbed with wet cotton swabs until it was removed. The skull was exposed by making an incision into the skin and gently pulling aside muscle. Metal retractors were used to keep the area exposed during surgery. The skull was cleaned and rubbed with a cotton swab dipped in hydrogen peroxide. Following stereotaxic measurements, the inferior colliculus (IC) was located and marked with a surgical pencil. A small 1 mm craniotomy was made with a surgical drill far away from the IC, above the frontal cortex. A small M1 screw was gently screwed into the small craniotomy for no more than two turns. A drop of surgical cyanoacrylate was placed to fasten the screw. Another craniotomy was made with a surgical drill above the inferior colliculus, the size did not exceed 2 mm × 2 mm and dura was

preserved. A drop of Dura-gel (Cambridge NeuroTech) was added to the craniotomy and the neural implant was lowered into the brain slowly. The implant was a custom-made16 channel (4 × 4) Microelectrode Array, 75 um platinum-iridium tip A, 0.75 MOhm impedance with each electrode of variable length to span the depth of the central nucleus of the IC (Figure S3). Once the implant was in place, Vaseline was used to cover any remaining space between the implant and the craniotomy. The ground wire was wrapped around the frontal screw. Then, the metal retractors were removed. Dental cement was applied to the implant and screw to ensure a strong bond. The cement was left to cure for 10 min and then the bat was removed from the anesthesia. The bats were allowed to recover in a cage over a heating blanket and monitored until they were awake and active. Then they were offered water and medicated using a needleless syringe with 1.0 mg/kg of meloxicam and Sulfatrim oral suspension 40 mg/mL: daily dose = 0.15 mL (330 mg/kg). Then the bats were returned to their home cage and offered food and water *ad libitum*. The bat was allowed to recover for 2 days to ensure the brain tissue had adapted to the implant and then the bat was used for chronic recordings. The microarray design yielded recordings from the dorsoventral, rostro-caudal and lateral IC. For each implanted bat, the implant was positioned to maximize sampling across the volume of the IC. Neural recording data were pooled to evaluate population responses.

Competitive foraging paradigm

The foraging paradigm consisted in having bats trained to eat from a small dish in a short period (10 min) at a restricted time of day on their own, and during testing sessions, bats would be paired to force a competitive situation. Generally, the bats were housed in butterfly cages (30 cm × 30 cm × 30 cm) with access to water and food *ad libitum*. Once the training of the animals began, food was removed from the home cage. During training, the bats were placed daily for 30 min each alone in the experimental butterfly cage (arena) within an anechoic room and offered food in a small glass dish that sat in the middle of the arena. Training involved encouraging the bats to approach the dish, either by moving the dish closer to them by hand and slowly moving it away or by using forceps to tease the bats with a mealworm and encourage them to move toward the plate. Training took up to 3 weeks for some bats, as they initially did not explore the cage on their own. The aim of the training was to ensure the bats immediately looked for food and associated the context with a feeding opportunity, but also to make sure they would reliably approach the dish immediately. As the bats learned to search for the food dish, the time in the experimental cage was reduced to 10 min sequentially. We considered a bat fully trained when they would immediately search for the dish upon being placed in the arena. The rationale for this was to ensure that competitive interactions would occur as both trained bats would approach the dish at the same time. The arena (Figure 1A) contained three ultrasonic custom-made Neu microphones from Ultrasound Advice connected to analog filters (bandpass 10–120 kHz), with a flat frequency response between 20 and 120 kHz (less than 5dBs differential). Audio recordings were acquired with an NI PCI DAQ card with a custom-made LabView software at a 250 kHz sampling rate. The reported measurement for the intensity of the calls is the average for each call across all three microphones. A MIRO high-speed video camera was used to collect behavioral data at 100 fps under IR lighting. After training was completed and the bats reliably approached the dish and ate within the 10-min window, 5 bats (3 females and 2 males) were implanted with chronic electrodes (Table S1). For each implanted bat we ran 3–6 recording sessions with a conspecific. The pairings are denoted on Table S1; some bats served as a pairing bat before being implanted for neural recordings in future trials. Each session consisted in plugging the bat to the head-stage and placing it in the arena, immediately followed by a nonimplanted conspecific and recording of electrophysiological data from the implanted bat and video and audio data from the interacting pair for 10 min. Electrophysiological was recorded at 16-bit precision and 40 kHz sampling rate using an OmniPlex D Neural Data Acquisition System recording system (Plexon, Inc.). After 10 min had passed, the bats were removed from the arena and returned to their home cage. Bats were weighed daily and observed to ensure that they had obtained sufficient food during the testing session. If for any reason, either of the bats didn't eat during the session, they were fed separately afterward and re-trained the day after.

QUANTIFICATION AND STATISTICAL ANALYSIS

Behavioral data was annotated using the software JWatcher and each bat in each video was tagged for behaviors in the following categories: echolocation (defined as the bat still or moving, moving its head and ears from side to side and having the mouth open), still (the bat appeared not moving any body part), grooming (the bats engaged in affiliative touching, all grooming observed was allogrooming), aggression (the bats pushed each other with the snouts, usually mouths were open and the bats propped themselves up on their thumbs/wings), feeding (the bats were actively chewing on mealworms) and locomoting (the bats were moving around the cage and not engaged in any of the other behaviors). Only one behavior was scored at a time. Deep Lab Cut[42] and webKnossos[39] were used to track the movement of bats across the arena and correlate to annotated behaviors. Acoustic data was first annotated using SASLab Pro to segment files into individual calls and remove background and chewing noises from the bats' eating. Later, a custom-made browser-based software (BattyKoda, Nunez-Mir et al. in preparation. https://github.com/angiesalles/battykoda) was used to classify the calls into distinct categories following the consolidated nomenclature proposed by Montoya and collaborators.[7] The identity of the caller cannot be discerned from our data. Spectrograms were potted using Kaleidoscope with FFT 256 and 50% overlap Hamming-window size 128. Neural data was first sorted using WaveClus[43] (Figure S2) and then analyzed with custom Python code. In that custom code, all the information was collated: The raw audio was loaded for amplitude measurements, which were translated into dB SPL using the supplied flat sensitivity curve from microphone manufacturer and the reference frequency of 41.4 kHz (equivalent to 1V/Pa). Then we used the standard reference of 20 μPa to calculate the sound pressure level (SPL) of the calls. The animal's tracked movement was used to measure traveled distance; the analyzed neural, behavioral and call data was sorted to relate spike counts, behavioral state, and communication calls. We identified an average of 40 muliunits per recording session and we evaluated spike number within a 25 ms window from call onset. This window was selected for consistency across calls, since the pooled

latency to peak of the PSTH for all calls was 10.1 ms (+- 6.9 ms) and the response returned to baseline at 24 ms. Responses to the same call types within a session were grouped to assess neural call selectivity. Population analysis of neural responses to calls in each behavioral state focused on neural responses to the acoustic stimuli, part of the analysis focused on all call types grouped, and for part of the analysis we calculated responses to specific call types. The selectivity index was calculated as the proportion of spikes a unit produced in response to a specific call type as compared to the spikes of that unit to all other call types. We have made all code for analysis available on GitHub. All comparisons were made using KruskallWallis nonparametric tests and Wilcoxon rank-sum tests. * represents $p < 0.05$.