

**Reaction of the Bat *Pipistrellus pipistrellus* to
Conspecifics and to Background Targets as
Documented by Synchronized Sound Recordings
and 3D Reconstructions of Flight Paths**

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List of abbreviations

A/D-converter	= Analog/Digital converter
bw	= bandwidth
cd	= call duration
dur	= duration
ER-vector	= vector from signal-emitting bat to signal-receiving bat
f end	= terminal frequency
FFT	= Fast Fourier Transformation
FM	= frequency-modulated
f peak	= peak frequency
int	= interval
PI	= pulse interval
QCF	= quasi constant-frequency
SPL	= sound pressure level
TOAD	= Time-Of-Arrival-Differences

Zusammenfassung

Fledermäuse haben bemerkenswerte Fähigkeiten. Sie sind die einzigen Säugetiere, die zum aktiven Flug fähig sind. Weiter verfügen sie über die Echoortung, mit der sie durch Aussendung von hochfrequenten Ortungslauten und Analyse der rückkehrenden Echos ihre Umgebung wahrnehmen. Durch diese Fähigkeit sind sie in der Lage, sich in völliger Dunkelheit zu orientieren, Beute und andere Objekte zu detektieren, zu lokalisieren und sogar zu charakterisieren. Für die erfolgreiche Nutzung dieser Fähigkeit spielt der Abstand zwischen den echoortenden Fledermäusen und umgebenden Strukturen eine entscheidende Rolle. Je nachdem, ob Fledermäuse weit weg von Hintergrundobjekten fliegen, wo es zu keiner Maskierung der Nutzechos von Beuteobjekten durch Störechos vom Hintergrund kommt (open space), sich in der Nähe von Objekten bewegen, wo die Fledermäuse ihre Ortungslaute anpassen, um eine Maskierung der Nutzechos durch Störechos zu vermeiden (edge space) oder sogar zwischen Hindernissen fliegen, in denen eine zeitliche Überlagerung von ausgesendeten Rufen und zurückkehrenden Nutz- und Störechos nicht vermieden werden kann (narrow space), senden sie speziell an diese drei grundlegend verschiedenen Habitattypen angepasste Ortungslaute aus. Im Lauf der Evolution haben sich verschiedene Jagdstrategien in Anpassung an die unterschiedlichen Habitattypen entwickelt, die gemeinsam die Grundlage für die Einteilung in sogenannte Gilden bilden. Eine dieser Gilden umfasst Fledermäuse, die im edge space fliegende Beute jagen und ihre Lautaussendungen dem jeweiligen Abstand zum Hintergrund anpassen („edge space aerial foragers“). Um erfolgreich jagen zu können, ist es für Fledermäuse dieser Gilde unabdingbar, eine Maskierung der Beuteechos durch Hintergrundechos (backward masking) oder durch das ausgesendete Echoortungssignal (forward masking) zu vermeiden. Die Fledermäuse müssen passende Echoortungsrufe aus ihrem Lautrepertoire auswählen, die kurz genug sind und sich hinsichtlich ihrer Frequenzstruktur eignen, um eine Maskierung zu vermeiden und um Beuteinsekten und andere Objekte genau lokalisieren zu können. Das Flug- und Echoortungsverhalten von Fledermäusen im edge space wurde bereits an mehreren Arten untersucht, jedoch blieben einige Fragen bisher unbeantwortet. Mit einem Mikrofonarray, auf dem mehrere Empfänger in definierten Abständen und einer festgelegten Anordnung zueinander angebracht waren, wurden die Laute freifliegender Zwergfledermäuse (*Pipistrellus pipistrellus*) aufgenommen. Durch Kreuzkorrelation der Laufzeitunterschiede zwischen den verschiedenen Mikrofonaufnahmen wurden die räumlichen Positionen von

individuellen Fledermäusen zu den Zeitpunkten ihrer Lautaussendungen berechnet. Dadurch war es möglich, Flugsituationen in Bezug auf den Abstand zwischen einzelnen Fledermäusen und Hintergrund sowie Interaktionen zwischen Fledermäusen mit deren inter-individuellen Abständen und Winkeln zu rekonstruieren und mit den in diesen Situationen ausgesendeten Lauten zu synchronisieren. Durch diesen methodischen Ansatz ist es gelungen, unser Wissen über das Echoortungsverhalten und Sozialverhalten von Zwergfledermäusen zu vertiefen und grundlegende Erkenntnisse über die Echoortung zu gewinnen.

Die vorliegende Arbeit besteht aus zwei Kapiteln. Sie knüpfen an meine Diplomarbeit an, in der ich mit der oben geschilderten Arraytechnik das Echoortungsverhalten von Zwergfledermäusen in räumlicher Nähe zu anderen Fledermäusen untersucht habe. Es hat sich gezeigt, dass fliegende Fledermäuse als bewegliche Objekte wahrgenommen werden und das Echoortungsverhalten in der gleichen Art in Abhängigkeit von der Distanz zu einem Objekt angepasst wird wie es bei unbeweglichen Objekten der Fall ist. Die Publikation dieser Studie wurde unter folgendem Titel veröffentlicht:

Götze, S. *et al.* No evidence for spectral jamming avoidance in echolocation behavior of foraging pipistrelle bats. *Sci. Rep.* **6**, 30978; doi: 10.1038/srep30978 (2016).

Im Verlauf der Untersuchungen im Rahmen der Diplomarbeit entdeckten wir Ultraschalllaute, die weder dem bisher bekannten Lautrepertoire noch dem Soziallautspektrum der Zwergfledermäuse zuzuordnen waren. Mit der fortgeschrittenen Methode der Arrayaufnahmen war es möglich, diese Laute eindeutig Zwergfledermäusen zuzuordnen und die Flugsituationen, in denen sie ausgesendet wurden, zu rekonstruieren. Es stellte sich heraus, dass Zwergfledermäuse zwei Typen von Soziallauten nutzen, um eine Futterquelle zu okkupieren und sie gegen Eindringlinge zu verteidigen. Die Studie über dieses Sozialverhalten ist bereits publiziert und bildet Kapitel 1 meiner Dissertation:

Götze, S., Denzinger, A. & Schnitzler, HU. High frequency social calls indicate food source defense in foraging Common pipistrelle bats. *Sci Rep* **10**, 5764 (2020)

Das zweite Kapitel bildet das Manuskript ‚*Pipistrellus pipistrellus* foraging in edge space adjust flight and echolocation behavior to prevent masking of potential prey‘, das demnächst für die Veröffentlichung eingereicht werden soll.

Wie alle hier genannten Studien wurde auch diese Untersuchung mit freifliegenden, jagenden Zwergfledermäusen an Straßenlampen durchgeführt. In dieser Studie wurde die

Grenze des äußeren edge space von *Pipistrellus pipistrellus* gemessen, wofür die Methodik der Flugwegsrekonstruktion und Zuordnung der Ortungslaute genutzt wurde. Anhand dieser Technik konnte genau bestimmt werden, ab welchem Abstand zu einer Straßenlampe und zum Boden sich die Lautparameter der Ortungslaute verändern. Die ab diesem Abstand stattfindende Verkürzung der Rufe dient dem Offenhalten eines akustischen Suchfensters, in dem nach Beute gesucht wird und in dem Beuteechos erwartet werden. Das Suchfenster würde sich bei längeren Rufen in Abhängigkeit von der Distanz zum Hintergrund schließen, wenn es zu einer zeitlichen Überlagerung zwischen ausgesendetem Ortungslaut und zurückkehrendem Beuteecho oder einer Überlagerung zwischen Beuteecho und Hintergrundecho kommen würde. Es wurde auch untersucht, ob es eine innere Grenze des edge space gibt. Tatsächlich gibt es einen von jagenden Fledermäusen nicht genutzten inneren Bereich (No-Forage-Area), in dem Zwergfledermäuse nicht mehr maskierungsfrei orten können, weil sich der ausgesendete Laut und das rückkehrende Hintergrundecho überlappen.

General Introduction

Guilds

Within the class Mammalia, bats belong to the truly outstanding order Chiroptera and are represented by a vast number of species with remarkable skills. Bats are the only mammals capable of powered flight and comprise ~1400 species of which ~1200 evolved the ability of laryngeal echolocation. The capability of ultrasonic signal emission and precise analysis of echoes reflected by their surroundings coupled with nocturnal activity enable them to occupy a wide range of ecological niches that are engaged by other species during daytime. As a result, bat species spread worldwide and can be found in almost all terrestrial areas except of the polar regions and high mountains. Chiropterans developed a rich diversity which is reflected by their various diets, foraging habitats, and foraging modes. We know bat species that feed on arthropods, pollen, fruit, nectar, also on fish, small vertebrates, and blood. Bats can forage for airborne prey such as flying insects, glean prey items from surfaces such as leaves or the ground and even trawl for fishes above water surfaces. In the course of evolution their flight and echolocation systems thereby adapted to the sensory and motor constraints they have to cope with depending on where, how and what they forage [1].

The distances between bat, food item, and background are the most relevant factors affecting the echolocation behavior and led to a classification of bat habitats into the three habitat types open, edge and narrow space. In open space, bats fly far enough from background structures that they do not suffer from masking of faint prey echoes by stronger background echoes, thus they do not react to the background in their echolocation behavior. In edge space, bats forage in the vicinity to background structures and react in their echolocation behavior to them by choosing appropriate echolocation calls from their call repertoire to prevent the masking of prey echoes by background echoes. In narrow space, bats forage within background structures such as dense vegetation, where a separation between prey echo and background echo in time is not possible. Narrow space foragers evolved alternative strategies to avoid masking, i.e., flutter detection.

Bats developed various foraging modes comprising active and passive strategies. Active strategies are characterized by signal emissions and the analysis of returning prey echoes, i.e., in aerial hawking or trawling, active gleaning or flutter detection, whereas bats

employing passive strategies rely on other sensory cues such as olfactory or acoustical stimuli to acquire food items, i.e., in passive gleaning.

Species that exploit the same class of environmental resources in a similar way developed similar adaptations, because they perform similar tasks when foraging [1]. They can be assigned to guilds, describing their task-specific adaptations. To date, we define seven bat guilds described by habitat type and foraging mode:

- open space aerial foragers
- edge space aerial foragers
- edge space trawling foragers
- narrow space flutter detecting foragers
- narrow space active gleaning foragers
- narrow space passive gleaning foragers
- narrow space active/passive gleaning foragers

Similar echolocation tasks defined by the habitat type and foraging mode led to a similar echolocation signal design and echolocation strategies within guilds, while the preferred prey items play a less important role.

The vespertilionid pipistrelles forage in open and edge space and are attributed to the guild of 'edge space aerial-hawking foragers'. The border between open and edge space is defined by changes in the echolocation behavior when bats cross the border between the two habitat types, since in edge space the bats react to background in their echolocation behavior, whereas in open space they do not. The border between open and edge space seems to be species-specific and was distinguished for *Vespertilio murinus* [2] and estimated for a small number of other species, although these values did not outgo a rather rough scale.

Echolocation in pipistrelle bats

Echolocating bats emit ultrasonic signals and analyse returning echoes to orient in space and to detect, characterize, and localize targets of interest [3]. The common pipistrelle bat *Pipistrellus pipistrellus* forages for airborne insect prey and emits single-element signals with characteristic downward sweeps consisting of broadband and narrowband elements [4]. The extent of these elements and further characteristic call parameters such as terminal frequency, duration, bandwidth, and pulse interval vary depending on the echolocation task

the bat is confronted with. Terminal frequencies of pipistrelles are characteristic and facilitate discrimination between closely related species. Those of *P.pipistrellus* have been found between 42 and 52 kHz and reflect also inter-individual differences. Their call durations were documented between 1 and 9 ms and bandwidth varied between 1 and 45 kHz at pulse intervals ranging from 5 to 180 ms. Call duration, terminal frequency and bandwidth are strongly correlated [5-7].

Two factors are crucial for the echolocation signal design: the behavioral situation of the bat and the distance of the bat to background. For the behavioral aspect, echolocation signals of foraging pipistrelles were categorized into search calls, and approach calls which include the calls of a long terminal group, the buzz, which is separated into buzz I and buzz II [4, 5, 8, 9]. Search and approach calls are distinguishable by significant differences in various call parameters. Search calls are emitted in search flight and transfer flight and serve the purpose of orientation and prey detection. They were measured with pulse interval means of 85-95 ms, bandwidth means of 7-31 kHz and duration means of 5.5-6.2 ms. After the detection of a target of interest pipistrelles start a pursuit and emit an approach sequence where call duration, sound pressure level (SPL), and pulse intervals are continuously reduced with decreasing target distance and where terminal frequencies are increased by 2-5 kHz compared to search calls. The buzz, a long terminal group, is emitted shortly before the capture maneuver. In the course of buzz I the pulse interval still decreases. Buzz II is characterized by a constant minimal pulse interval of 5-6 ms and by a characteristic drop in frequency at the transition from buzz I to buzz II (Fig.1).

Beside these call parameters, that identify the behavioral situation of a foraging bat, the echolocation signals of *P. pipistrellus* are linked to distinct behaviors. In search flight, pipistrelles move their heads more or less up and down and left and right, which is discernible by alternating amplitudes in single microphone recordings and was well documented [7]. This 'scanning behavior' with the rather directional acoustic beam is comparable with the movement of torch's light beam in darkness and leads to an increase of the bat's search cone volume where it can search for and await prey [5, 7]. In contrast, in approach flight an orientation towards the target of interest by turning the head and ears towards the prey was reported by Kalko (1995).

In the terminal phase, pipistrelles alter their body position to prepare for an insect catch, a behavior which was also well described and documented by Kalko (1995) [5].

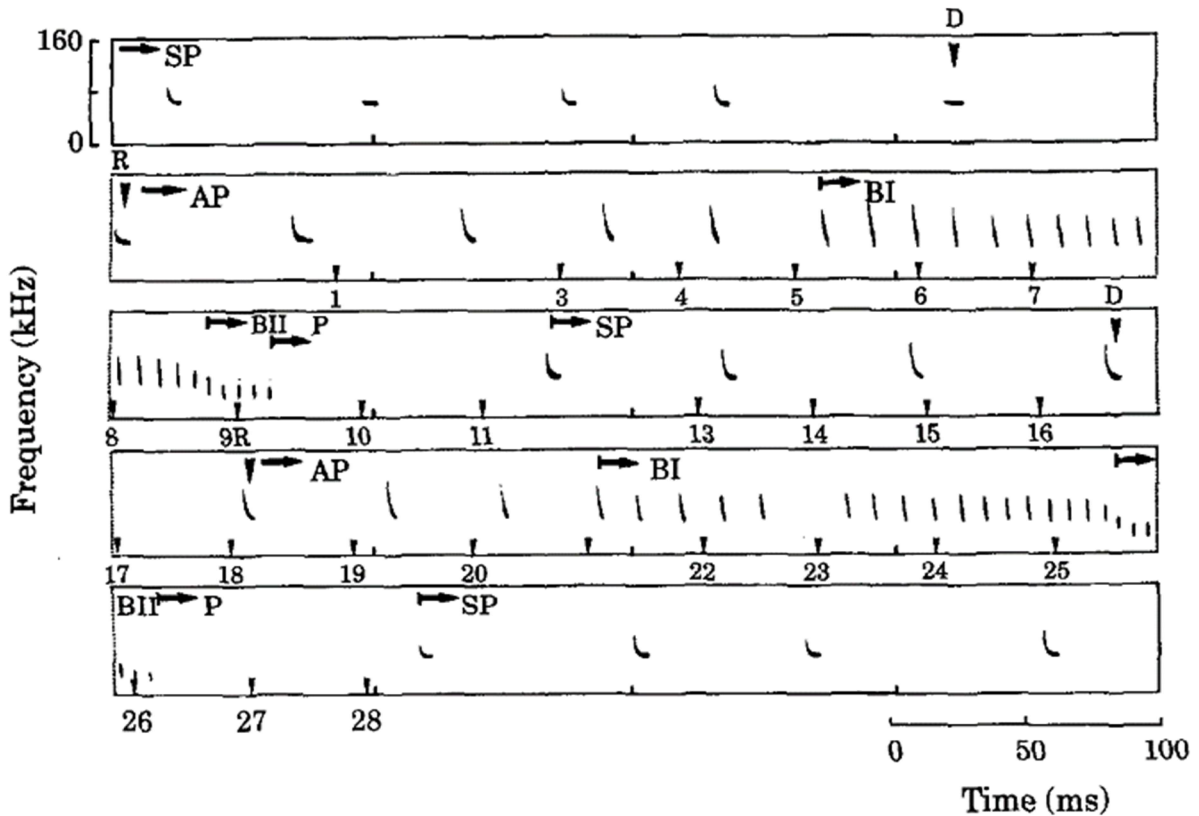


Fig.1: Echolocation sequence of *Pipistrellus pipistrellus* with signals of the search phase (SP), detection of insect (D) and reaction (R), approach phase (AP) with terminal phase comprising buzz I (BI) and buzz II (BII) followed by a pause (P). Adapted from Kalko (1995) [5].

Search call design also varies according to the surroundings and therefore the distance between echolocating bat and sound reflecting structures. Bats choose echolocation calls from an internal repertoire which contains signals ranging in the case of *P.pipistrellus* from short, broadband signals with high terminal frequencies to long, narrowband signals with low terminal frequencies. In open space, where bats fly so far apart from clutter that they do not react to the background in their echolocation behavior [10], they emit narrowband, quasi-constant frequency (QCF) signals with minor bandwidths and mean durations of 6 ms at rather high SPL and pulse intervals of ~ 98 ms [7]. It is proposed that such signals evoke a higher neuronal activity in the relevant frequency-selective filters of the bats' auditory system than frequency-modulated (FM) signals which activate the filter only for a short time thus enabling the detection of weak echoes of insects over longer distances [3]. In contrast, when the bats forage in the vicinity to background clutter, their search calls are characterized by shorter duration (mean 3 ms) and they start with a broadband, frequency-modulated component that sweeps down to a lower QCF-part. The SPL is reduced and the pulse intervals are shorter (~ 89 ms) compared to the signals emitted in uncluttered space [4,

7]. These broadband calls are well adapted for the localization and classification of prey items [11, 12].

Bats use echolocation not only to search for prey, but also to orient and to navigate in space, and to avoid collisions. Emitted echolocation signals are reflected by prey items, but also by other surrounding structures. These echoes are important for bats to determine their spatial position. However, depending on the duration of the echolocation signal and the distance between bat, prey, and background, such background echoes could mask the weaker prey echoes and are therefore named clutter echoes.

A prey item, that is so close to a bat, that its reflected echo overlaps with the emitted signal (forward masking), is located within the 'signal overlap zone' and a successful perception of the prey echo by the bat is unfeasible. A similar problem occurs when the prey item is located so close to the background, that it is positioned within the 'clutter overlap zone', where its echo overlaps with the echo of the background (backward masking). A prey item can only be successfully detected when it is located within an 'overlap free window' between signal and clutter overlap zone, where emitted signal, prey echo and background echo are separated in time. The width of the signal and the clutter overlap zone depends on signal duration. 1 ms of signal duration accounts for 0.17 m width of the signal and the clutter overlap zone. A signal with duration of 6 ms therefore accounts for a signal overlap zone and clutter overlap zone of each 1.02 m and would result in a closed overlap-free window at a distance of 2.04 m and less between bat and background.

To overcome the problem of masking, bats developed a strategy comprising an adaptation of their echolocation behavior in edge space to keep the overlap-free window open while the distance to background diminishes. Decreased signal durations reduce the width of the overlap zones and thereby increase the overlap free window by 0.34 m per 1 ms. An emission of shorter echolocation signals helps to keep the overlap-free window open and enables the bats to forage closer to background structures without acoustic masking (Fig.2).

Furthermore, 'spatial unmasking' in the hearing of bats has been reported for *Eptesicus fuscus*, which describes a reduced sensitivity for echoes reaching the bats ears from directions off axis. A diminished perception of clutter echoes from angles off their echolocation direction may help bats to separate prey echoes coming in from ahead and clutter echoes coming in from aside by a flight path orientation along background structures, keeping clutter echoes beyond a certain angle [13, 14].

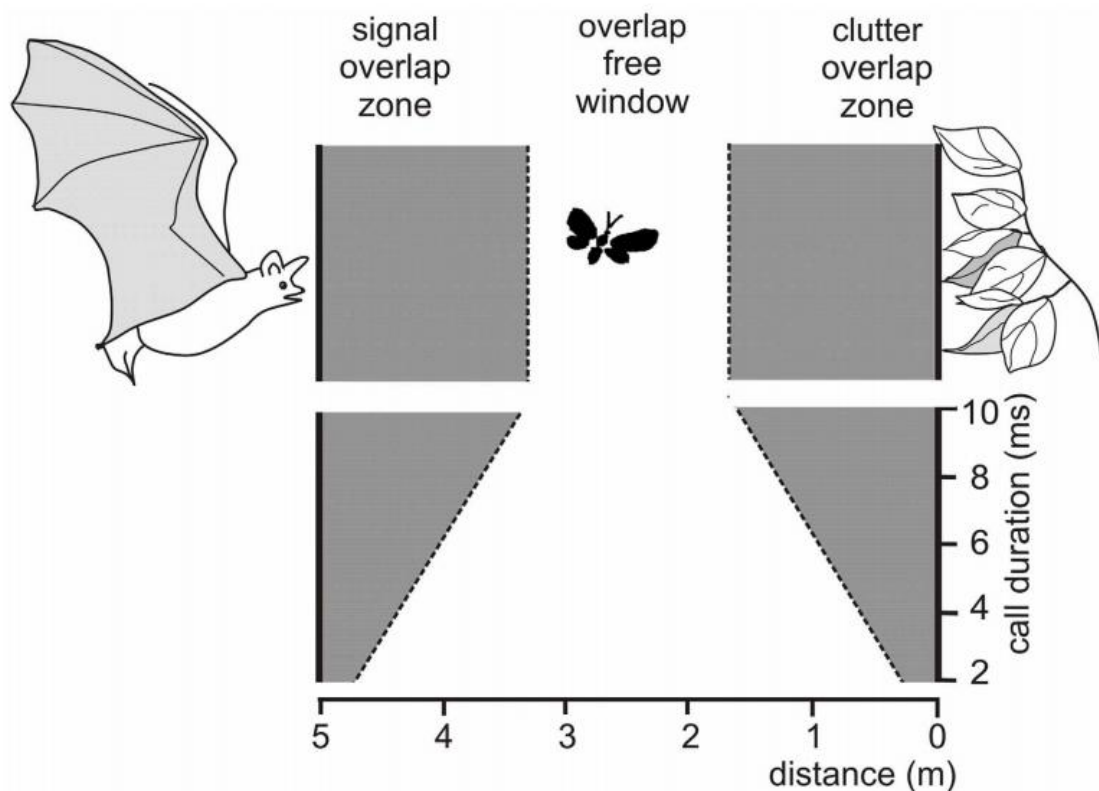


Fig.2: Schematic drawing illustrating the conditions for overlap between emitted signal, prey echo and background echoes a bat encounters when foraging at a distance of 5 m to vegetation. The prey echo overlaps with the emitted signal when an insect flies in the signal overlap zone and with the clutter echoes reflected by the background when it flies in the clutter overlap zone. In the overlap-free window no overlap occurs. The width of the overlap zones depends on signal duration. At durations between 10 and 2 ms, each overlap zone ranges between 1.70 and 0.34 m, if a speed of sound of 340 m/s is assumed. A reduction of signal duration by 1 ms reduces the width of each overlap zone by 0.17 m and thus increases the width of the overlap-free window by 0.34 m. Adapted from Denzinger and Schnitzler (2013) [1].

Social calls and social interactions in bats

Bats hold a variety of calls which have a social function and coordinate the behavior of bats. These social calls differ from echolocation calls and their frequencies reach from ultrasonic to humans hearing range. Their multifaceted designs differ throughout bat species and according to behavioral context, but based on general structural features they were classified into four groups (Type A,B,C,D) [15]. Social calls are emitted in particular behavioral situations, which have been attributed to the groups of structural design. Type A (squawk) and Type B (repeated, trill) social calls are emitted exclusively by stationary bats and play a role in aggressive behavior (Type A) or express increased irritation ('distress calls', Type B) [15]. Type C calls are emitted in mother-infant interactions and function as 'isolation

calls' during flight and in the roost. Type D calls are characterized by a complex, song-like structure and have been documented as a part of mating and territorial behavior in flying bats. In both cases, the emitter occupies a certain area to acquire food or mating partners and competes for these resources with conspecifics. However, the underlying intentions of Type D call emission have been interpreted in oppositional ways as either an expression of attraction or repulsion [16-21].

Social calls are emitted in situations where individual bats interact with each other. In non-flying bats such interactions can be documented by video recordings which are synchronized with sound recordings. Such recordings allow judging of the behavioral situation of the interacting bats and the spatial relations between them. The social calls of flying bats are also directed to other individuals. However, so far it was not possible to evaluate the behavioral situation and the spatial relations of the interacting bats as it was not possible to document their flight paths in synchronization with their vocal behavior.

Aims and method of the thesis

The main aim of my thesis was to study how *Pipistrellus pipistrellus* reacts to conspecifics and to background targets in its vocal behavior when foraging under natural conditions. These studies were only possible with a method that allowed a synchronized determination of the flight and vocal behavior of free flying bats.

For this purpose we constructed a multichannel recording system following a design by scientists of the University of Southern Denmark [22], which was composed of three ultrasonic microphones arranged on a stationary array at different positions and defined distances to an embedded reference microphone. This setup facilitated audio recordings of the bats' signal emissions and together with an AD-converter with high sampling rate a conversion into digital data as a prerequisite for the subsequent analysis of call parameters. Concurrently, the recording system provided the opportunity to reconstruct the spatial positions of the bats in the moment of signal emission. Synchronous recordings by microphones at different positions and defined distances to a reference microphone record each signal with differences in times of arrival between the channels according to the distances between bat and each microphone, respectively. These time lags encode the spatial position of the bat in the moment of signal emission. Reconstructed positions for consecutive signals of individual bats were assembled to individual flight paths and allowed

an assignment of emitted signals to actual flight situations. Furthermore, the spatial reconstructions provided a basis for the calculation of distances and angles between individual bats and between bats and background structures.

The distances between bat and background structures are crucial for the use of echolocation and directly affect the echolocation and flight behavior of bats. Former studies revealed that echolocation signal parameters change depending on the distance between bat and background and led to the definition of the habitat types narrow, edge and open space. . These studies referred to stationary background structures such as buildings vegetation and the ground [2, 4, 23-25]. However, aerial-hawking insectivorous bats like *Pipistrellus pipistrellus* often forage at food patches such as insect swarms around street lights [26], where flight paths of multiple foraging individuals are likely to converge. Bats are regularly confronted with situations in which other bats move within their vicinity and collisions must be avoided, be it at feeding spots or when leaving the roost. Detailed knowledge about the echolocation behavior of bats moving in groups or in vicinity to other bats was yet limited. A certain amount of studies about the echolocation behavior of bats flying with conspecifics postulated a Jamming Avoidance Response (JAR), comprising frequency shifts in their echolocation calls to avoid a potential confusion about returning echoes [27-33]. We hypothesized, that the documented frequency changes in the echolocation behavior of bats flying in groups occurred due to spatial vicinity to other bats, because flying bats are perceived by other bats as moving obstacles and collisions must be prevented by an appropriate echolocation behavior which is comparable to that in edge space around stationary obstacles.

The analysis of synchronized recordings of echolocation and flight behavior of foraging bats confirmed this hypothesis. Bats experience other bats as moving targets and react to them depending on their inter-individual distance and angle with changes of their call parameters in the same way as it is described for stationary obstacles. In the vicinity of other bats which are ahead of the reacting bat they emit echolocation calls of decreased duration and increased bandwidth and terminal frequency to prevent the masking of target echoes by their own emitted signals (forward masking) and facilitate an accurate localization of the moving object for collision avoidance. The results of this study were already published in the context of my diploma thesis:

Götze, S. *et al.* No evidence for spectral jamming avoidance in echolocation behavior of foraging pipistrelle bats. *Sci. Rep.* **6**, 30978; doi: 10.1038/srep30978 (2016).

In the course of this study we became aware of ultrasonic signals of noticeable high frequency that were neither part of the known species-specific echolocation call repertoire nor the known social call repertoire of the relevant species. They appeared in temporal proximity to complex social calls and during social interactions between conspecifics. That raised the question, if these calls were social calls and if they were connected in any way to complex social calls. The study of these 'high frequency calls' became the first chapter of my dissertation. We hypothesized that high frequency calls are agonistic social calls and are emitted in the presence of conspecifics for the purpose of food source defense. Furthermore we hypothesized, that complex social calls neither are emitted as agonistic nor mating calls as proposed by former studies, but serve as an announcement of the emitter's presence. The investigation of the two call types together with the associated flight behaviors of involved bats was conducted with the microphone array, which facilitated synchronous audio recordings and determination of the bats spatial relations by flight path reconstruction. The results of this study are presented in the published paper

Götze, S., Denzinger, A. & Schnitzler, HU. High frequency social calls indicate food source defense in foraging Common pipistrelle bats. *Sci Rep* **10**, 5764 (2020)

in Chapter 1.

The second part of my dissertations deals with the species-specific border between open and edge space in *Pipistrellus pipistrellus*, which was so far only estimated visually. With multichannel recordings by the microphone array the outer border of edge could be determined on a fine-grained scale using the advanced method of flight path reconstruction and call assignment. It was also investigated whether there is an inner border of edge space, too, which foraging bats would not pass. We hypothesized that flight and echolocation behavior of *Pipistrellus pipistrellus* foraging in edge space has the function to keep their search space free of masking.

The results of this study are presented in the manuscript '*Pipistrellus pipistrellus* foraging in edge space adjust flight and echolocation behavior to prevent masking of potential prey' in Chapter 2 and is prepared for submission.

1. Denzinger, A. and H.U. Schnitzler, *Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats*. Front Physiol, 2013. **4**: p. 164.
2. Schaub, A. and H.U. Schnitzler, *Echolocation behavior of the bat Vespertilio murinus reveals the border between the habitat types "edge" and "open space"*. Behavioral Ecology and Sociobiology, 2006. **61**(4): p. 513-523.
3. Schnitzler, H.U. and E.K.V. Kalko, *Echolocation by insect-eating bats*. Bioscience, 2001. **51**(7): p. 557-569.
4. Kalko, E.K.V. and H.U. Schnitzler, *Plasticity in echolocation signals of European pipistrelle bats in search flight: implications for habitat use and prey detection*. Behavioral Ecology and Sociobiology, 1993. **33**(6): p. 415-428.
5. Kalko, E.K.V., *Insect pursuit, prey capture and echolocation in pipistrelle bats (Microchiroptera)*. Animal Behaviour, 1995. **50**(4): p. 861-880.
6. Götze, S., et al., *No evidence for spectral jamming avoidance in echolocation behavior of foraging pipistrelle bats*. Sci Rep, 2016. **6**: p. 30978.
7. Seibert, A.M., et al., *Scanning behavior in echolocating common pipistrelle bats (Pipistrellus pipistrellus)*. PLoS One, 2013. **8**(4): p. e60752.
8. Melcón, M.L., A. Denzinger, and H.-U. Schnitzler, *Aerial hawking and landing: approach behaviour in Natterer's bats, Myotis nattereri (Kuhl 1818)*. Journal of Experimental Biology, 2007. **210**(24): p. 4457-4464.
9. Melcón, M.L., H.-U. Schnitzler, and A. Denzinger, *Variability of the approach phase of landing echolocating greater mouse-eared bats*. Journal of Comparative Physiology A, 2009. **195**(1): p. 69-77.
10. Schnitzler, H.-U., C.F. Moss, and A. Denzinger, *From spatial orientation to food acquisition in echolocating bats*. Trends in Ecology & Evolution, 2003. **18**(8): p. 386-394.
11. Ostwald, J., H.-U. Schnitzler, and G. Schuller, *Target discrimination and target classification in echolocating bats*, in *Animal sonar*. 1988, Springer. p. 413-434.
12. Moss, C.F. and H.-U. Schnitzler, *Behavioral studies of auditory information processing. Hearing by bats*, 1995: p. 87-145.
13. Sümer, S., A. Denzinger, and H.-U. Schnitzler, *Spatial unmasking in the echolocating Big Brown Bat, Eptesicus fuscus*. Journal of Comparative Physiology A, 2009. **195**(5): p. 463-472.
14. Warnecke, M., et al., *Spatial release from simultaneous echo masking in bat sonar*. The Journal of the Acoustical Society of America, 2014. **135**(5): p. 3077-3085.
15. Pfalzer, G. and J. Kusch, *Structure and variability of bat social calls: implications for specificity and individual recognition*. Journal of Zoology, 2003. **261**(1): p. 21-33.
16. Sachteleben, J. and O. von Helversen, *Songflight behaviour and mating system of the Pip pipistrellus in an urban habitat*. Acta Chiropterologica, 2006. **8** (2): p. 391-401.
17. Lundberg, K. and R. Gerell, *Territorial Advertisement and Mate Attraction in the Bat Pipistrellus-Pipistrellus*. Ethology, 1986. **71**(2): p. 115-124.
18. Gerell, R. and K. Lundberg, *Social-Organization in the Bat Pipistrellus-Pipistrellus*. Behavioral Ecology and Sociobiology, 1985. **16**(2): p. 177-184.

19. Barlow, K.E. and G. Jones, *Function of pipistrelle social calls: Field data and a playback experiment*. *Animal Behaviour*, 1997. **53**: p. 991-999.
20. Miller, L.A. and H.J. Degn, *The acoustic behavior of four species of vespertilionid bats studied in the field*. *Journal of comparative physiology*, 1981. **142**(1): p. 67-74.
21. Skiba, R., *Europäische Fledermäuse*. Westarp Wissenschaften, Hohenwarsleben, 2003.
22. Surlykke, A., S. Boel Pedersen, and L. Jakobsen, *Echolocating bats emit a highly directional sonar sound beam in the field*. *Proceedings of the Royal Society B: Biological Sciences*, 2009. **276**(1658): p. 853-860.
23. Jensen, M.E. and L.A. Miller, *Echolocation signals of the bat *Eptesicus serotinus* recorded using a vertical microphone array: effect of flight altitude on searching signals*. *Behavioral Ecology and Sociobiology*, 1999. **47**(1): p. 60-69.
24. Siemers, B.M. and H.-U. Schnitzler, *Natterer's bat (*Myotis nattereri* Kuhl, 1818) hawks for prey close to vegetation using echolocation signals of very broad bandwidth*. *Behavioral Ecology and Sociobiology*, 2000. **47**(6): p. 400-412.
25. Rydell, J., *Variation in the sonar of an aerial-hawking bat (*Eptesicus nilssonii*)*. *Ethology*, 1993. **93**(4): p. 275-284.
26. Rydell, J., *Exploitation of Insects around Streetlamps by Bats in Sweden*. *Functional Ecology*, 1992. **6**(6): p. 744-750.
27. Bates, M.E., S.A. Stamper, and J.A. Simmons, *Jamming avoidance response of big brown bats in target detection*. *J Exp Biol*, 2008. **211**(Pt 1): p. 106-13.
28. Gillam, E.H., N. Ulanovsky, and G.F. McCracken, *Rapid jamming avoidance in biosonar*. *Proc Biol Sci*, 2007. **274**(1610): p. 651-60.
29. Habersetzer, J., *Adaptive Echolocation Sounds in the Bat *Rhinopoma-Hardwickei* - a Field-Study*. *Journal of Comparative Physiology*, 1981. **144**(4): p. 559-566.
30. Obrist, M.K., *Flexible Bat Echolocation - the Influence of Individual, Habitat and Conspecifics on Sonar Signal-Design*. *Behavioral Ecology and Sociobiology*, 1995. **36**(3): p. 207-219.
31. Surlykke, A. and C.F. Moss, *Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory*. *J Acoust Soc Am*, 2000. **108**(5 Pt 1): p. 2419-29.
32. Ulanovsky, N., et al., *Dynamics of jamming avoidance in echolocating bats*. *Proc Biol Sci*, 2004. **271**(1547): p. 1467-75.
33. Ratcliffe, J.M., et al., *Conspecifics influence call design in the Brazilian free-tailed bat, *Tadarida brasiliensis**. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 2004. **82**(6): p. 966-971.

Structure of the thesis

My thesis is a study about the vocal behavior of free flying bats under natural conditions. I investigated the reactions to conspecifics and to background targets by the synchronization of audio recordings and flight path reconstructions of foraging common pipistrelles.

The thesis is subdivided into two chapters. Chapter 1 is already peer-reviewed and published in the journal 'Scientific Reports', Chapter 2 is prepared as manuscript for publication.

In the first chapter I described a type of social call that was so far not identified as a component of the species' social call repertoire and I scrutinized another social call type, which was already described, but interpreted in two oppositional ways. The flight behavior of interacting pipistrelles was synchronized with their vocal behavior and discussed against the background of present literature. Chapter 1 is already published:

Götze, S., Denzinger, A. & Schnitzler, H.U. High frequency social calls indicate food source defense in foraging Common pipistrelle bats. *Sci Rep* **10**, 5764 (2020)

In the second chapter I studied the echolocation behavior of foraging bats around street lights. I measured the distance around street lights at which echolocation behavior was adapted to background and at which the distance between bat and street light could no further be reduced. The study is presented in Chapter 2 with the title '*Pipistrellus pipistrellus* foraging in edge space adjust flight and echolocation behavior to prevent masking of potential prey', which is prepared as manuscript by the authors Götze, S., Denzinger, A. and Schnitzler, H.U..

Author information and contribution from others to chapter 1

The study was planned by Prof. Dr. Hans-Ulrich Schnitzler, Dr. Annette Denzinger and me, I conducted the audio recordings, extracted the echolocation parameters and reconstructed the flight paths. I analysed and edited the data and wrote the first draft of the manuscript. Prof. Dr. Hans-Ulrich Schnitzler, Dr. Annette Denzinger and I interpreted the data, cooperated in writing the manuscript and I published the paper with them as co-authors. Technical resources were provided by Prof. Dr. Hans-Ulrich Schnitzler and Dr. Annette Denzinger.

Publication of the results of chapter 1

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High frequency social calls indicate food source defense in foraging common pipistrelle bats

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Abstract

Social calls have the function to coordinate the behavior of animals. In the presence of conspecifics foraging common pipistrelle bats (*P. pipistrellus*) emitted, in addition to typical echolocation signals, two types of social calls: complex social calls and an as-of-yet undescribed, short, frequency-modulated call type with high terminal frequency, which we term 'high frequency social call'. By recording the flight and acoustic behavior of free flying pairs of foraging *P. pipistrellus* with an array of four microphones we were able to determine their three-dimensional flight paths and attribute emitted calls to particular behavioral situations. complex social calls were emitted at further inter-individual distances and at large bearing angles to conspecifics, whereas high frequency social calls were produced at significantly shorter distances and at smaller bearing angles. These calls were associated with chasings and the eviction of the intruder. We assume that the emission of both types of social calls by foraging bats reflects a two-stage-process of the occupation and defense of a food patch. Common pipistrelle bats use complex social calls to claim a food patch and switch to agonistic behaviors, including chasings and high frequency social call emissions, when they defend their foraging territory against an intruder.

Introduction

Acoustic signaling plays an important role in animal communication¹. Among mammals, bats exhibit a remarkable repertoire of social calls, including aggression and distress signals between conspecifics and for the defense against predators, signals in mother-infant interactions as well as advertisement calls for mating²⁻⁵.

In this study, we focused on the social call repertoire of foraging *Pipistrellus pipistrellus*, one of the most well-studied vespertilionids of Europe. This insect-eating bat emits signals for

orientation, detection and localization of airborne prey^{6,7}, and produces social calls for intraspecific and interspecific communication. Its echolocation and social signal design have been investigated and described by numerous studies, although their social call repertoire has yet to be fully described or understood^{2,8–15}.

It has been established that pipistrelles use four Types (A-D) of social calls², which differ considerably in structural design. In contrast to Types A and B, which are emitted only by stationary bats at the roost, Types C and D are produced by individuals during flight. Of these flight calls, Type C is a single-element contact signal used in mother-infant interactions, whereas Type D consists of 2–5 multiharmonic frequency-modulated syllables, a call that is also referred to as ‘complex social call’^{2,16}. Studies investigating the behavioral function of complex social calls have found two different contexts in which they are emitted – as part of courtship flightsongs for mating, and in foraging behavior^{3,11,15,17} – which is surprising given the opposing intentions (attraction and repulsion) intended by these two behaviors². Complex social calls in both of these behavioral situations appear indistinguishable from their structure, although they show slight but significant differences¹². These might be caused by individual variations, since Pfalzer and Kusch² showed that individual bats can be distinguished by intraspecific differences in call duration and frequency. In fact, due to these individual signatures, bats may be able to identify and distinguish individual conspecifics^{18–21}. The question remains, however, of how a bat could distinguish between incoming complex social calls that were either meant to attract (‘advertisement calls’^{2,11,22}, ‘mating calls’² or ‘songflight calls’¹²) as compared to those meant to repel (‘agonistic calls’)¹⁰. It has been suggested that various cues could serve to differentiate foraging and courtship flightsongs, such as the number of complex social calls in a series¹⁰, diverging pulse intervals^{8,10,12}, season²³, location¹³, and/or flight behavior^{10,13} of the emitter. Here, we challenge the differentiation between advertisement and agonistic calls, and rather hypothesize that complex social calls at all times serve as an announcement of a bat’s presence. If complex social calls are broadcasted by a bat at a foraging site, the signal should have a repelling effect on male and female conspecifics, because it signals the presence of a resident and the occupation of a food resource. The same type of calls can also act as an attracting signal on females and as a repellent signal for males during mating season, because the announcement of presence is then linked with the

occupation of a mating territory. For simplicity, we chose the term 'Type 1 call' for complex social calls for the course of this study.

In the current study we also recorded signals from *Pipistrellus pipistrellus* which were different from the species' echolocation signals and their known social calls. These were sweeps of short duration and high terminal frequency which were emitted in striking temporal proximity to Type 1 calls. We preliminary termed these 'Type 2 calls' and sought to understand the purpose of their emission.

We assumed that not only Type 1 but also Type 2 calls have a social function and tested the following hypotheses:

1. Type 2 calls are social calls and have an impact on the behavior of conspecifics.
2. Type 2 calls serve as agonistic social calls and elicit the eviction of intruders.

Methods

Species and study sites. We recorded free flying common pipistrelles (*Pipistrellus pipistrellus*) from May to October in 2010 and from 2013 to 2015. The recordings were conducted during 34 nights at 16 different sites in Brandenburg, East Germany, and Baden-Württemberg and Bavaria, South Germany. Two recording sites in Baden-Württemberg were illuminated by street lights; 14 sites were unlit. The illuminated recording sites were positioned underneath street lights at the periphery of residential areas, whereas the unlit sites were situated along forest edges and water resources such as lakes and rivers. Recordings started 30–50 minutes after sunset, lasting 1 hr 45 min on average. On individual nights the location was changed after 2 hours for additional recordings. We recorded a total of more than 57 hrs of audio, of which 37 hrs 38 min were analyzed. All recording sites consisted of edge space habitats along structures and were connected to open space habitats. All recordings were conducted in accordance with relevant guidelines and regulations.

Sound recordings. In the course of this study we successively used two horizontally-oriented T-shaped and planar microphone arrays of equal design but different sizes. Each array consisted of four microphones pointing upwards in the same direction; three microphones were positioned in a line and a fourth was fixed at a right angle to the central microphone.

All outer microphones were placed at equal distance from the central microphone. The array used in 2010 consisted of four Knowles microphones (Model SPM0404UD5) with outer microphones at distances of 1 m from the central microphone. From 2013 to 2015, we used a larger array with custom-made microphones fixed at a distance of 2 m from the central microphone.

The arrays were positioned at ground level, or in the case of uneven ground, adjusted to heights of 1.2–1.6 m above ground. Each sound recording lasted 20 seconds and was amplified, digitized and stored as a .wav file using the custom-made software 'Battery'. In 2010, the sound recordings were digitized with a sampling rate of 250 kHz with an A/D-converter of Type USB-6251 (National instruments, Texas). From 2013 to 2015 the sampling rate was increased to 400 kHz due to enhanced technical equipment (National instruments, A/D-converter of Type USB-6356).

Data analysis. During the sampling period, we recorded a total of 2078 Type 1 calls and 952 Type 2 calls. We visualized the recorded signals as color spectrograms (FFT 512, Blackman window, dynamic range of 70 dB) using custom-made software (Selena, University of Tübingen, Germany). The spectrograms were plotted with a temporal resolution of 0.06 ms and a spectral resolution of 156.3 Hz due to auto-padding and time interpolation. The beginning and end of signals were measured in the spectrograms using the automatically applied criterion of -15 dB below highest amplitude. We measured several signal parameters, including duration, peak frequency, terminal frequency, bandwidth, and pulse interval and tested each for normal distribution using a Kolmogoroff-Smirnoff-Test (JMP 11.2.0). Terminal frequencies of echolocation calls, Type 1 and Type 2 calls of equal duration were compared by an ANOVA (JMP 11.2.0). Given that the data points for pulse intervals were not normally distributed, we chose median, and first (Q1) and third quantile (Q3) as a measurement for pulse intervals for all comparisons. We tested the impact of both call types on pulse intervals between search calls using a Wilcoxon test (JMP 11.2.0).

Signals of insufficient amplitude were still used for reconstruction of flight paths when possible. In addition, we counted Type 1 and Type 2 calls within our recordings over four years from May to October and calculated the emission rate for each call type per each 10-minute recording sequence.

Differentiation of single flights and flights of two or more bats. Typically, echolocation calls from a single bat flying above the array produced a sequence of steady signals characterized by constant or smoothly changing amplitude and pulse intervals of approximately 90 ms; in some cases, however, an approach sequence with decreasing pulse intervals and durations was emitted, often ending in a feeding buzz. Terminal frequencies of echolocation signals have a negative correlation with sound duration, which was considered when using frequency for the identification of individuals.

Audio recordings of more than one bat flying within the recording area were characterized by irregular pulse intervals of less than 90 ms. Individual bats often differed in their individual terminal frequencies for distinct call durations, by which they could be distinguished.

For the description of the social interaction behavior, we classified the bat that was foraging continuously around a street light (at illuminated sites) or was consistently present within the range of our microphones (at unlit sites) as 'resident'. A second bat that then entered the space was classified as 'intruder', since it entered a foraging site where the resident was already present.

3D-flight path reconstruction. By using microphone arrays for audio recordings, we were able to reconstruct the flight paths and subsequently the flight behavior of recorded bats. Calls of bats flying above the T-shaped array reached the four microphones at different times due to different distances between the emitter and the receivers. These differences in arrival time encoded the spatial positions of the bats in each moment of signal emission relative to the reference microphone, which was part of the array. Time-Of-Arrival-Differences (TOAD) were determined by measuring the time lag at which the cross-correlation function between the signal received by the reference microphone and the signals received by each of the other three microphones reached the maximum value. By using the three calculated TOADs we could determine the three-dimensional positions of the bats above the array in each moment of signal emission. The positions from consecutive calls of individual bats were then assembled and smoothed using a moving average filter for nine consecutive calls to estimate continuous individual flight paths, providing individual coordinates for each millisecond of the recording. We labeled different signal types (echolocation calls, Type 1 and Type 2 calls) with different colors for their discrimination in results. For the calculation of TOADs and the reconstruction of flight paths we used the

software Matlab R2016a (Math Works, Massachusetts, USA) and designed appropriate programs.

We compared the spatial relations between bats during emissions of Type 1 calls and Type 2 calls. For the calculation of the bearing angles between a bat emitting a Type 1 or Type 2 call and the bat receiving the Type 1 or Type 2 call, we used the spatial position of the emitter in the moment of signal emission and its interpolated spatial position 1 ms before the call emission to create a flight direction vector. This vector was set to be the flight direction with an angle of 0° . The spatial position of the bat receiving the call was taken from its interpolated flight path at the point of time the call was emitted. The bearing angle was measured between the flight direction vector and the vector from the emitter's position to the receiver's position at call emission (ER vector, Fig. 1).

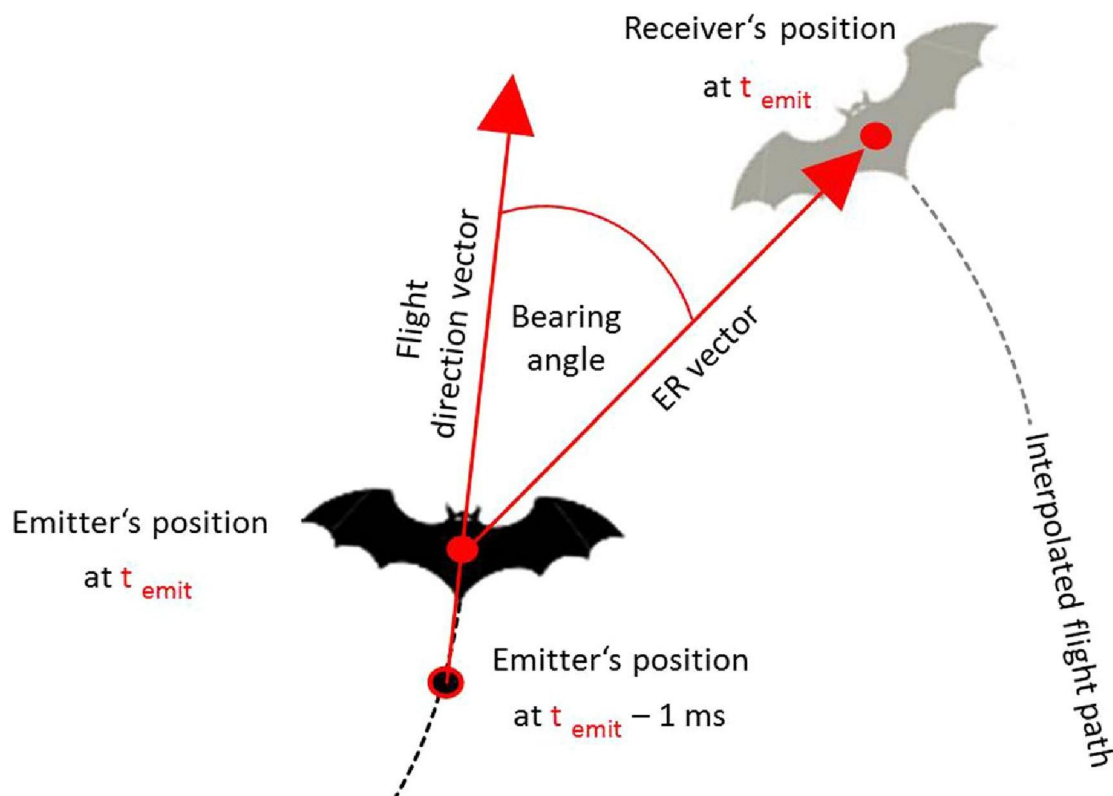


Figure 1. Determination of bearing angle and inter-individual distance between emitter and receiver. The bearing angle was measured between the flight direction vector of the emitter (black bat) and the ER vector between emitter and receiver (grey bat) in the moment of call emission (t_{emit}). The flight direction vector was defined by the spatial position of the emitter at t_{emit} and its interpolated spatial position 1 ms earlier.

Differences in inter-individual angles for Type 1 and Type 2 call emissions were compared by an ANOVA (JMP 11.2.0). We also determined the inter-individual distance between the two bats in the moment of Type 1/Type 2 call emission. Distances were not normally distributed and log-transformed before conducting an ANOVA (JMP 11.2.0).

Results

Type 1 and Type 2 call emissions by *Pipistrellus pipistrellus*. We reconstructed flight paths of 12 audio recordings of foraging residents and intruders of *P. pipistrellus* containing 60 Type 1 calls and 74 Type 2 calls. In 11 of 12 recordings with Type 2 calls, both call types were present, whereas in one recording Type 1 did not accompany a Type 2 call. In 8 of 12 recordings, Type 1 calls were emitted shortly before Type 2 calls, with a median interval from beginning of Type 1 call to beginning of Type 2 call of 510 ms (Q1: 310 ms, Q3: 930 ms, $n = 20$, Fig. 2). Across all recordings, Type 1 and 2 were emitted exclusively by the resident with the exception of one case, where an intruder also emitted a Type 1 call.

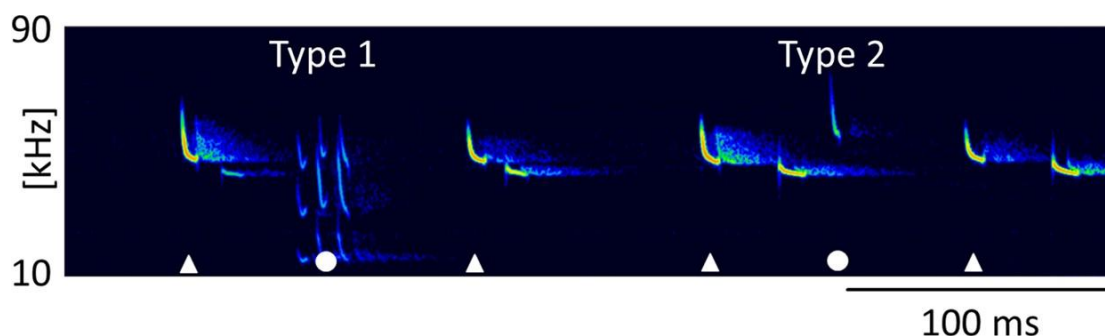


Figure 2. Type 1 and Type 2 call between echolocation signals. Type 1 call and Type 2 call emitted by a resident in mutual flight with an intruder. Calls of the resident are marked by white symbols, with Type 1 and 2 calls (circles) interspersed between search calls (triangles).

Type 1 calls. Type 1 calls of *P. pipistrellus* consisted of 3–5 multiharmonic and frequency-modulated syllables with terminal frequencies of 16.0 ± 0.8 kHz (mean \pm SD, $n = 50$). These complex calls lasted on average 25 ms (25.0 ± 3.9 ms, mean \pm SD, $n = 50$) and were interspersed between echolocation signals. The number of echolocation calls between Type 1 calls varied from 2 to 83 signals within discrete recording sequences of 20-second duration. The social calls were emitted during foraging flight, indicated by feeding buzzes and typical pursuit behavior.

Type 2 calls. Type 2 calls were short, frequency-modulated calls with terminal frequencies above the terminal frequencies of echolocation signals of the emitter. They were interspersed between echolocation signals and delivered as either single calls, or groups of two or three consecutive calls (Fig. 3a–c).

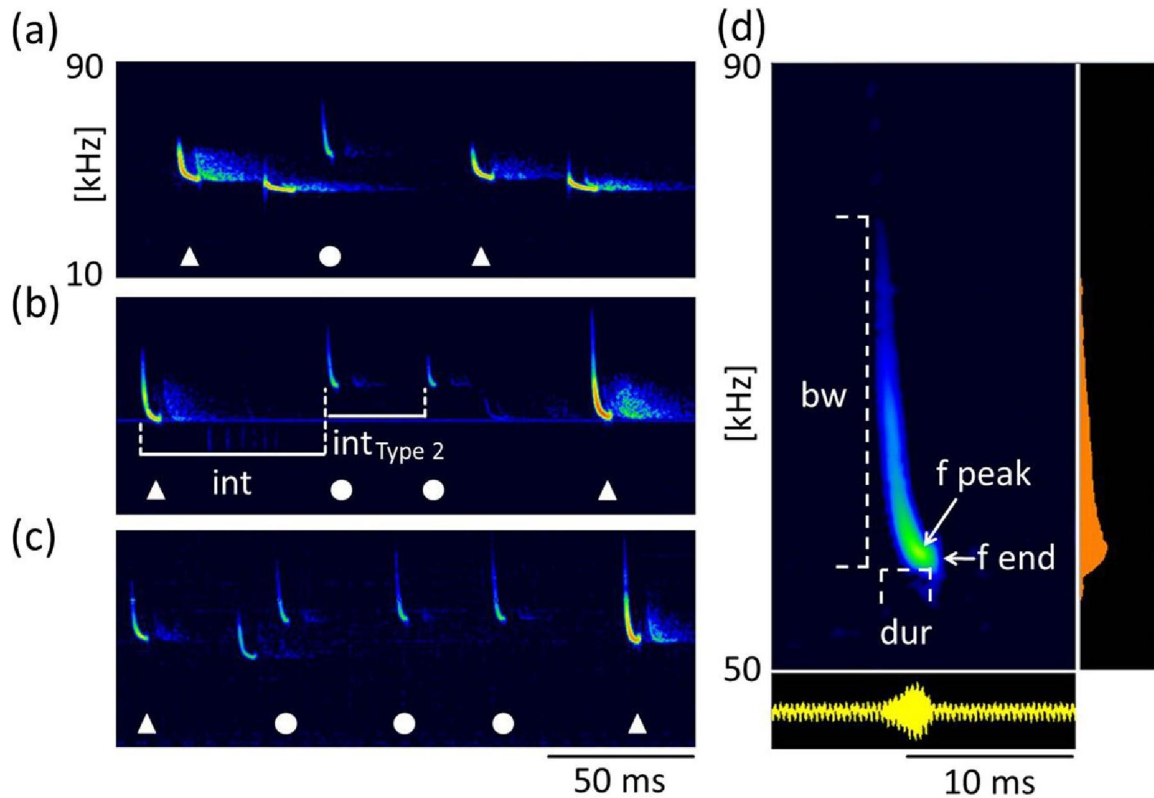


Figure 3. Type 2 calls. Type 2 calls were emitted by the resident in presence of an intruder as single calls (a) or in groups of two (b) or three (c) consecutive calls. Calls of the resident are marked by white symbols, with Type 2 calls (circles) interspersed between search calls (triangles). Call parameters for characterization of Type 2 calls are bandwidth (bw), duration (dur), peak frequency (f peak) and terminal frequency (f end), (d). Pulse intervals of Type 2 calls were measured to the preceding search call (int, b) and within grouped Type 2 calls (int Type 2, b).

These calls were emitted by the resident and were recorded exclusively in presence of an intruder. A single recording revealed an emission in presence of an interspecific intruder (sp. *Pipistrellus nathusii*). The terminal frequency of Type 2 calls ranged from 51.3 to 60.4 kHz (56.7 ± 2.1 kHz, mean \pm SD, $n = 27$) and was 4.5 to 13.9 kHz (8.8 ± 2.2 kHz mean \pm SD, $n = 27$) higher than the terminal frequency of the preceding echolocation call by the same individual. Analysis of terminal frequency and duration for all Type 2 calls and search calls that fulfill the criterion of sufficient amplitude revealed significantly higher terminal

frequencies (57.0 ± 2.2 kHz, Mean \pm SD, $n = 14$) for Type 2 calls than search calls (48.7 ± 1.5 kHz, Mean \pm SD, $n = 35$, Anova, $F(1,47)=232.13$, $p < 0.0001$) of comparable duration (2.7–3.7 ms, Fig. 4). Type 2 calls had peak frequencies of 58 ± 2.4 kHz (mean \pm SD, $n = 73$), bandwidths of 6.4 ± 1.7 kHz (mean \pm SD, $n = 14$) and durations of 3.3 ± 0.3 ms (mean \pm SD, $n = 14$, Table 1).

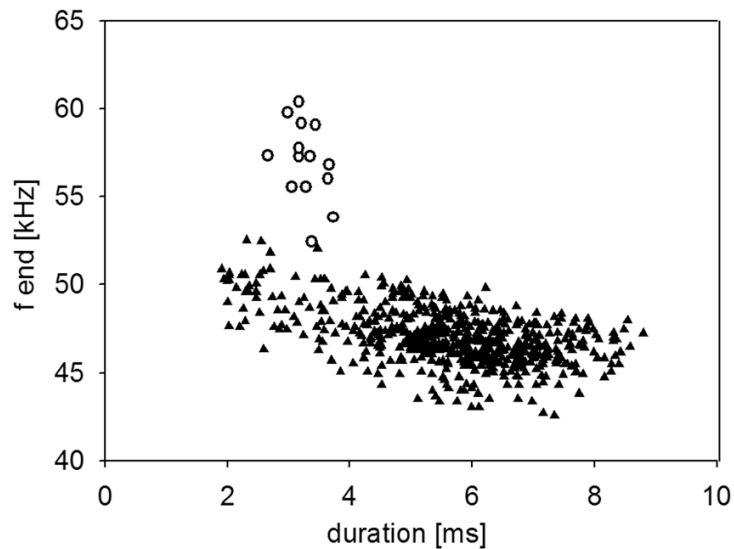


Figure 4. Correlation of terminal frequency (f_{end}) of Type 2 calls and search calls with call duration. Type 2 calls (white circles) have higher terminal frequencies than search calls (black triangles).

	duration [ms]	f peak [kHz]	bandwidth [kHz]	f end [kHz]
n	14	73	14	26
mean	3.3	58.0	6.4	56.7
SD	0.3	2.4	1.7	2.2
min	2.7	52.1	4.2	51.3
max	3.7	64.3	9.3	60.4

Table 1. Parameters of Type 2 calls.

Pulse intervals. In search flight, *P. pipistrellus* emitted echolocation signals with durations of up to 10 ms and pulse intervals around 95 ms (Median 95.4 ms, Q1: 80.8 ms, Q3: 107.6 ms, $n = 2355$). Pulse intervals of ~ 90 ms indicate sound emissions most likely in rhythm with wing beat, whereas a pulse interval of ~ 180 ms indicates a wing beat without sound emission.

Type 1 and Type 2 calls were interspersed between echolocation signals and their emission affected the pulse intervals of echolocation calls in the following way:

The median pulse interval from the beginning of Type 1 calls to the beginning of the preceding echolocation call was 46.8 ms (Q1: 44.2 ms, Q3: 51.4 ms, n = 60) and from the beginning of Type 1 calls to the beginning of the following echolocation call was 85.2 ms (median, Q1: 76.8 ms, Q3: 102.8 ms, n = 58). Resulting from the insertion of a Type 1 call, the pulse intervals between echolocation signals increased to median values of 133.6 ms (Q1: 124.2 ms, Q3: 154.2 ms; n = 59, Wilcoxon, $p < 0.0001$).

Type 2 calls were interspersed with median intervals of 53.5 ms (Q1: 45.8 ms, Q3: 60.1 ms, n = 58) to the preceding echolocation call. However, in contrast to Type 1, the interspersed Type 2 calls did not always affect the pulse intervals of echolocation signals. Single interspersed Type 2 calls induced no extension of pulse intervals between echolocation signals (median 96.9 ms, Q1: 89.4 ms, Q3: 121.5 ms, n = 43, Wilcoxon, $p = 0.0572$), groups of two Type 2 calls induced extended pulse intervals between echolocation calls of 152.1 ms (median, Q1: 144.9 ms, Q3: 162.5 ms, n = 12, Wilcoxon, $p < 0.0001$) and a group of three consecutive Type 2 calls induced a median pulse interval of 169.4 ms between the preceding and following echolocation call of the emitter.

Pulse intervals of grouped Type 2 calls lasted 34.2 ms (median, Q1: 29.2 ms, Q3: 39.3 ms, n = 14).

Flight behavior. *Type 1 calls.* Type 1 calls were emitted by the resident in single flight and in flights with an intruder (Fig. 5a,b). Flight path reconstructions revealed flights along constant routes, interrupted by turning patterns with feeding buzzes, as usually observed in foraging bats. No false landings or gliding elements such as those reported from songflights were observed. The resident often emitted Type 1 calls shortly before a conspecific (or occasionally an individual of a different species) intruder emerged.

Type 2 calls. Type 2 calls were emitted only by the resident and exclusively in the presence of an intruder (Fig. 5b). Flight paths of residents and intruders revealed that Type 2 call emission mostly took place either during chases of the intruder by the resident (60%) or in frontal encounters of both bats (25%). In both flight situations the resident flew directly towards the intruder. Few Type 2 calls were emitted when flight paths already crossed (15%) and were still in close proximity. Analysis of consecutively-taken audio recordings revealed that intruders left the recording area after receiving Type 2 calls from the resident (Fig. 6).

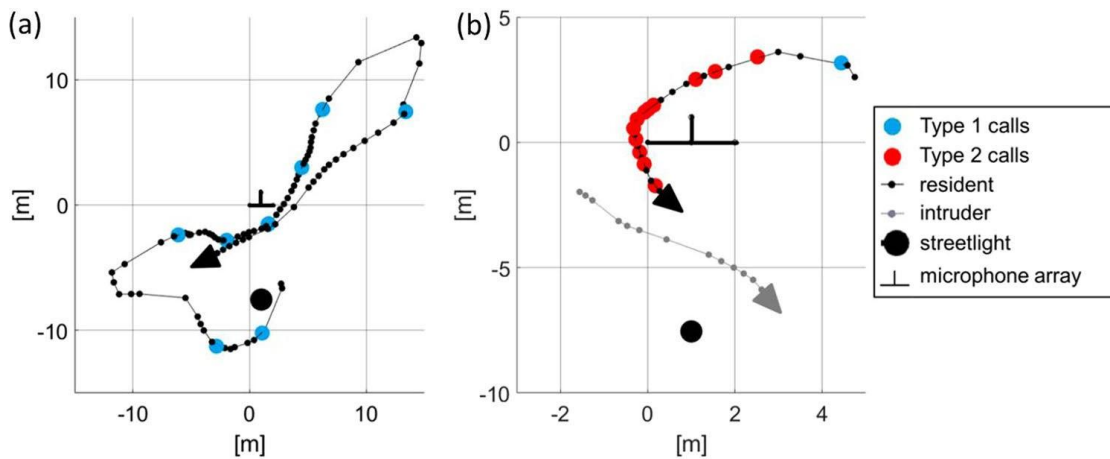


Figure 5. Top view of the flight paths of a foraging resident in single flight (a) and in flight with an intruder (b). Small dots indicate positions where echolocation signals were emitted by the resident (black) or by the intruder (grey). Large dots indicate Type 1 and Type 2 signals. Type 2 calls (red dots) were emitted exclusively by the resident in presence of an intruder, often during chase flights.

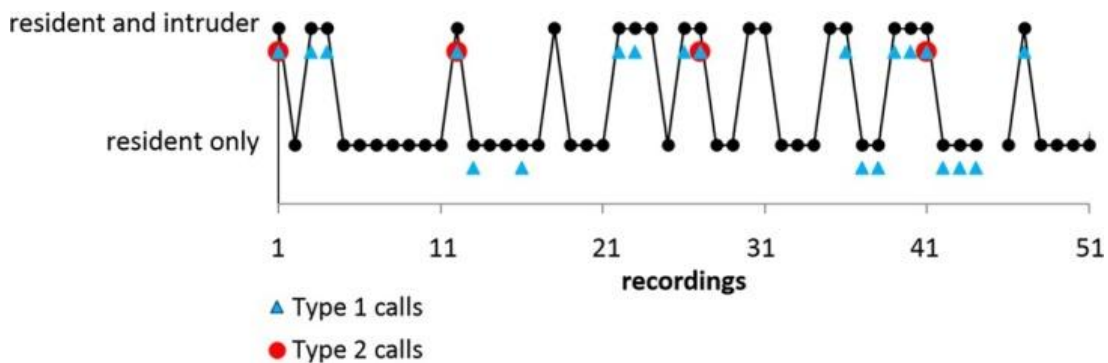


Figure 6. Emission pattern and effect of Type 2 calls. Example of vocal behavior of a resident individual foraging alone or in the presence of intruders documented by 50 consecutive recordings each with a duration of 20 seconds. Recordings are interrupted by approx. 0.9 seconds for data saving by the recording device (total evaluation time 18 minutes). Black dots indicate that the resident only or both resident and intruder were present as evidenced by their echolocation signals. Recordings where the resident emitted one or more Type 1 calls are marked by blue triangles, recordings with Type 2 calls are indicated by red circles. The resident emitted Type 2 calls exclusively in presence of intruders. After the emission of Type 2 calls intruders left the foraging site and were not traceable during the subsequent audio recording.

Bearing angle and inter-individual distance between resident and intruder at Type 1 and Type 2 call emission. During Type 1 call emission, the bearing angles to the intruder varied widely, with a range from 23.9 to 173.5°. The values for the same measurement at Type 2

call emission were less variable and ranged from 5.3 to 112.2°. Bearing angles to the intruder were significantly smaller during Type 2 call emissions ($42.1 \pm 3.6^\circ$, median \pm SEM, $n = 68$) than during Type 1 call emissions ($76.8 \pm 6.5^\circ$, median \pm SEM, $n = 36$; Anova, $F(1,102) = 26.82$, $p < 0.0001$, Fig. 7a, b). Inter-individual distances between resident and intruder were also significantly smaller during Type 2 call emissions (6.5 ± 0.6 m, median \pm SEM, 1.1–23.8 m, min-max, $n = 68$) than during Type 1 call emissions (12.0 ± 0.8 m, median \pm SEM, 1.5–21.1 m, min-max, $n = 36$; Anova, $F(1,102) = 11.74$, $p = 0.0009$, Fig. 7a, c).

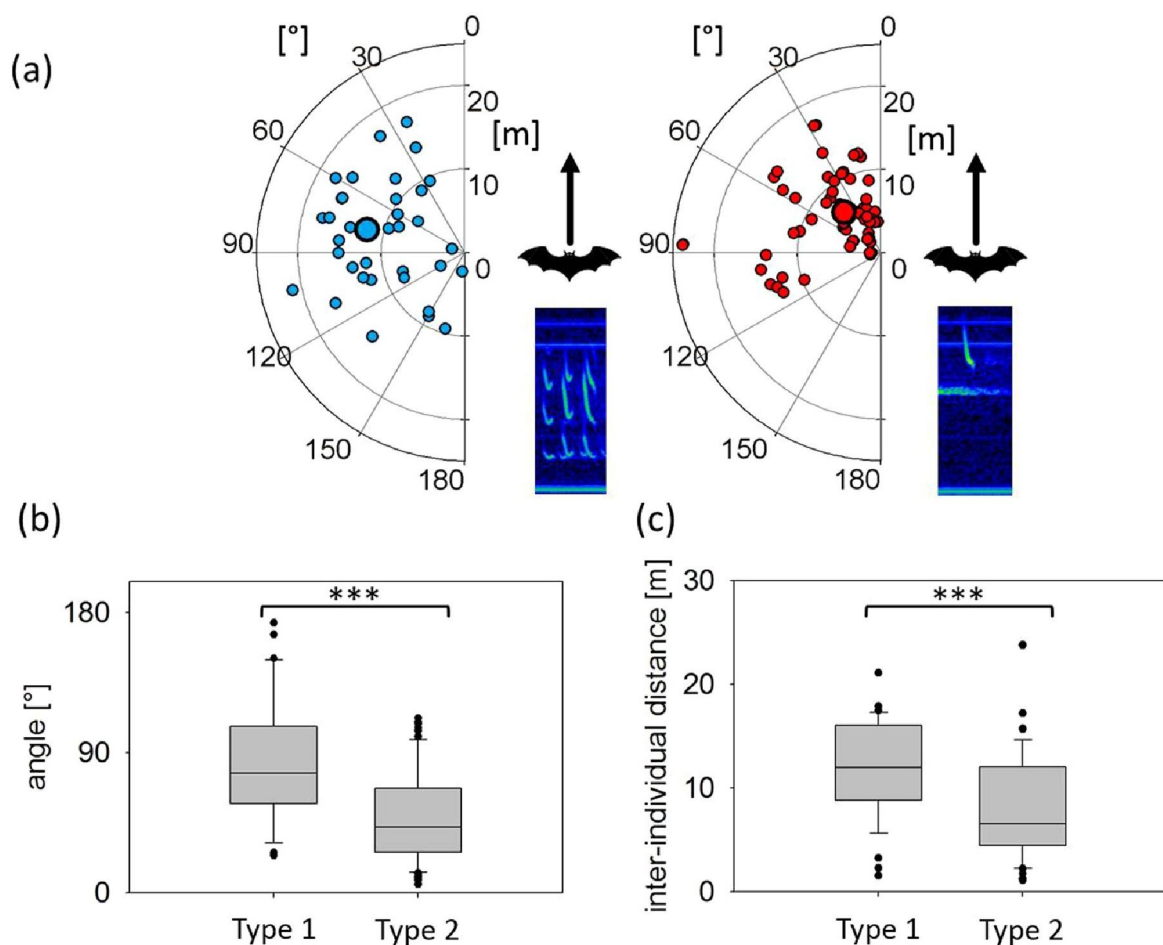


Figure 7. Spatial positions of intruders in relation to the position and flight direction of the resident at the emission of Type 1 and Type 2 calls. Spatial positions of intruders (small circles) at Type 1 call emission (left) and Type 2 call emission (right) by the resident with median position (large circles) indicated by distance and angle relative to the resident's position and flight direction (0°) (a). Comparison of relative angles (b) and distances (c) at the emission of Type 1 ($n = 36$) and Type 2 calls ($n = 68$) revealed significant differences. Bearing angles and inter-individual distances were smaller at Type 2 call emissions than for Type 1 call emissions.

Annual distribution of social calls. Type 1 and Type 2 calls were recorded throughout the entire activity season from May to October (Table 2). As shown by the annual distribution, Type 1 call emission was evenly distributed over 82% of all recording nights, whereas Type 2 was emitted in 45% of nightly samples and occurred only in detached nights in July, August and September. The highest rates of Type 1 and Type 2 calls were recorded in October.

month	n Type 1 calls / hour	n Type 2 calls / hour	n recording hours	n recording nights	n recording nights with Type 1 calls	n recording nights with Type 2 calls
May	25.2	37.6	4.6	5	4	3
Jun	15.5	1.8	7.6	4	4	2
Jul	44.1	43.7	6.2	4	3	1
Aug	18.9	6.8	8.1	4	3	1
Sep	31.7	3.7	5.7	3	2	1
Oct	236.5	77.6	5.5	2	2	2

Table 2. Annual distribution of Type 1 and Type 2 call emissions.

Discussion

Our sound recordings with an array of four microphones allowed the reconstruction of the three-dimensional flight paths of foraging pipistrelles flying either alone or together with conspecifics and the calculation of the bats' positions at the emission of the recorded sounds. With this method we are able to attribute the emission of specific calls to particular behavioral situations. In addition to echolocation signals, we recorded two other types of calls from foraging common pipistrelles. One type, termed 'Type 1 call', was described in former publications and identified as social call^{2,11,12,15-17}. The other type, termed 'Type 2 call', had yet to be described, but we hypothesize based on the study presented here that it too serves a social function (see below).

The social call 'Type 1' consists of 2–5 multi-harmonic frequency-modulated syllables and was named 'complex social call' by Pfalzer and Kusch (2003). We will adopt this term for the discussion of our data. Complex social calls have been found in sound recordings from foraging bats but also in recordings from pipistrelle males while performing so-called 'songflights' in their mating territory¹⁰. Sound sequences consisting of echolocation signals

with interspersed complex social calls have been named flightsongs by Smotherman et al. 2006, and both foraging and courtship flightsongs^{3,10,11,14,15,17,24} have been interpreted as a notable example of territorial singing^{3,10,12,13,16,25,26}. Many studies have attempted to identify functional differentiation between complex social calls emitted during courtship and foraging, e.g. by naming them advertisement or agonistic calls^{2,9–13,24,27,28}. Arguments for this differentiation are based on observations that complex social calls attracted females to mating grounds but were also observed in courtship and foraging flightsongs emitted at sites where chasing behavior also occurred. Our data appears to resolve this apparent discrepancy at least for foraging *Pipistrellus pipistrellus*, by attributing the recorded signal types to specific behavioral situations.

Flightsongs were emitted by foraging residents independently from the presence or absence of conspecifics. However, in situations where we only recorded the resident, this individual may have sensed the echolocation calls of an approaching intruder and reacted with social call emission before our recording equipment picked up the conspecific's signals. In situations with two bats, the reconstructed flight paths of both individuals revealed that exclusively the resident emitted complex social calls. Only once, an approaching intruder emitted a complex social call, while the resident oscillated between two street lamps. We assume this was an attempt to occupy the territory around the streetlight while the resident was at the neighboring lamp. In this case, however, the resident returned and repelled the intruder. Complex social calls were always emitted before chasing the conspecific away from the food source. In contrast to reports from former studies, we show that the complex social calls were emitted only prior and not during chasings and therefore do not support an agonistic function. If a conspecific was traceable, the inter-individual distance and the bearing angle between resident and intruder were rather high, revealing an undirected broadcast of the signal. This supports our hypothesis that complex social calls serve as a general announcement of the emitter's presence and display territorial behavior. Our findings here support the conclusions of former studies, that bats emit complex social calls as a warning to intruders^{11,29} and that foraging flightsongs have a territorial character^{3,30}. If these warnings were disregarded by intruders and conspecifics entered the claimed territory, the resident reacted with agonistic behavior and chased these individuals away from the food source. During these chases the resident emitted Type 2 calls, which we term 'high frequency social calls' based on their frequency structure. Single or groups of high

frequency social calls were interspersed between echolocation signals and were emitted at short distances between intruder and resident and at minor bearing angles, indicating they were specifically addressed to the intruder. Their reduced range caused by increased sensitivity to atmospheric attenuation due to their high frequency also supports this conclusion. Both behaviors, chasings in combination with high frequency social call emission, preceded the intruder's departure. This supports the hypothesis that high frequency social calls have a repelling effect on the behavior of conspecifics and are true agonistic social calls. The attribution of complex and high frequency social calls to specific behavioral situations showed that the occupation and defense of a food source is a two-stage process: A resident produces foraging flightsongs with complex social calls to claim a resource and to warn conspecifics of entering its territory (claiming phase). The disregard of this warning leads to an escalation where the resident defends the food source by reacting to the intruder with chasings and accompanied high frequency social calls (agonistic phase).

Flightsongs can also occur as part of courtship behavior, when bats lay claim to a mating territory^{11-13,23}. It is not surprising, then, that complex social calls in foraging and courtship flightsongs do not essentially differ in structure, but allow the individual recognition of a resident by individualized signal design^{2,12,16,31}. Complex social calls are likely to transmit information such as species, sex, age, weight and size of their sender and qualify other bats to estimate or even recognize the characteristics of the territory holder. This may enable female bats to choose a mating partner and help avoiding costly agonistic interactions between intruders and residents.

The incidence of flightsongs and high frequency social calls during the activity season of *P. pipistrellus* reflects phases of low food availability in spring and fall as well as the mating season in late summer. The number of complex social calls increases when insect density decreases^{11,29,32,33}, with time after dusk^{34,35}, or due to falling air temperatures³⁶, hence when competition for food increases. Under such circumstances, the claiming of a food patch may be necessary for residents to avoid potential agonistic interactions with other bats. High frequency social calls were recorded primarily in May and October, when weather conditions are poor and insect activity is considerably reduced. Since insects are scarce at these times of the year, bats might engage in riskier behaviors more often by intruding into already occupied territories and eliciting agonistic food source defense behaviors by the resident. According to our data, high frequency social calls also occurred

more often in July, but only in one of four recording nights. In this case, a decrease in insect activity due to unfavorable weather conditions may have led to higher competition for food. Our results suggest that high frequency social calls have an agonistic function and serve to repel other bats from food sources when competition for food is high. Our work suggests that bats use complex social calls for claiming resources such as courtship or foraging territories. However, these calls are not necessarily associated with agonistic behavior. Whether high frequency social calls are also emitted by males for the defense of their mating territories requires further investigation.

References

1. Bradbury, J. W. & Vehrencamp, S. L. *Principles of animal communication*. (1998).
2. Pfalzer, G. & Kusch, J. Structure and variability of bat social calls: implications for specificity and individual recognition. *Journal of Zoology* **261**, 21–33, <https://doi.org/10.1017/s0952836903003935> (2003).
3. Smotherman, M., Knornschild, M., Smarsh, G. & Bohn, K. The origins and diversity of bat songs. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **202**, 535–554, <https://doi.org/10.1007/s00359-016-1105-0> (2016).
4. Chaverri, G., Ancillotto, L. & Russo, D. Social communication in bats. *Biological Reviews* **93**, 1938–1954 (2018).
5. Bohn, K. M. & Gillam, E. H. In-flight social calls: a primer for biologists and managers studying echolocation. *Canadian Journal of Zoology* **96**, 787–800 (2018).
6. Schnitzler, H. U. & Kalko, E. K. V. Echolocation by insect-eating bats. *Bioscience* **51**, 557–569, [https://doi.org/10.1641/0006-3568\(2001\)051\[0557:ebieb\]2.0.co;2](https://doi.org/10.1641/0006-3568(2001)051[0557:ebieb]2.0.co;2) (2001).
7. Schnitzler, H.-U., Moss, C. F. & Denzinger, A. From spatial orientation to food acquisition in echolocating bats. *Trends in Ecology & Evolution* **18**, 386–394, [https://doi.org/10.1016/s0169-5347\(03\)00185-x](https://doi.org/10.1016/s0169-5347(03)00185-x) (2003).
8. Kühn, M. *Die Balzlaute der Zwergfledermaus *Pipistrellus pipistrellus**, Eberhard-Karls-University Tübingen, (2018).
9. Budenz, T., Heib, S. & Kusch, J. Functions of bat social calls: the influence of local abundance, interspecific interactions and season on the production of pipistrelle (*Pipistrellus pipistrellus*) type D social calls. *Acta Chiropterologica* **11**, 173–182, <https://doi.org/10.3161/150811009x465794> (2009).
10. Sachteleben, J. & von Helversen, O. Songflight behaviour and mating system of the pipistrelle bat (*Pipistrellus pipistrellus*) in an urban habitat. *Acta Chiropterologica* **8**, 391–401, [https://doi.org/10.3161/1733-5329\(2006\)8\[391:Sbamso\]2.0.Co;2](https://doi.org/10.3161/1733-5329(2006)8[391:Sbamso]2.0.Co;2) (2006).
11. Barlow, K. E. & Jones, G. Function of pipistrelle social calls: Field data and a playback experiment. *Animal Behaviour* **53**, 991–999, <https://doi.org/10.1006/anbe.1996.0398>

- (1997).
12. Barlow, K. E. & Jones, G. Differences in songflight calls and social calls between two phonic types of the vespertilionid bat *Pipistrellus pipistrellus*. *Journal of Zoology* **241**, 315–324 (1997).
 13. Lundberg, K. & Gerell, R. Territorial Advertisement and Mate Attraction in the Bat *Pipistrellus-Pipistrellus*. *Ethology* **71**, 115–124 (1986).
 14. Gerell, R. & Lundberg, K. Social-Organization in the Bat *Pipistrellus-Pipistrellus*. *Behavioral Ecology and Sociobiology* **16**, 177–184, <https://doi.org/10.1007/Bf00295153> (1985).
 15. Miller, L. A. & Degn, H. J. The acoustic behavior of four species of vespertilionid bats studied in the field. *Journal of comparative physiology* **142**, 67–74, <https://doi.org/10.1007/BF00605477> (1981).
 16. Jahelková, H., Horáček, I. & Bartonička, T. The advertisement song of *Pipistrellus nathusii* (Chiroptera, Vespertilionidae): a complex message containing acoustic signatures of individuals. *Acta Chiropterologica* **10**, 103–126, <https://doi.org/10.3161/150811008x331144> (2008).
 17. Skiba, R. Europäische Fledermäuse. *Westarp Wissenschaften, Hohenwarsleben* (2003).
 18. Gelfand, D. L. & McCracken, G. F. Individual variation in the isolation calls of Mexican free-tailed bat pups (*Tadarida brasiliensis mexicana*). *Animal Behaviour* **34**, 1078–1086 (1986).
 19. Balcombe, J. P. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. (1990).
 20. Jones, G., Hughes, P. & Rayner, J. The development of vocalizations in *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during post-natal growth and the maintenance of individual vocal signatures. *Journal of Zoology* **225**, 71–84 (1991).
 21. Boughman, J. W. Greater spear-nosed bats give group-distinctive calls. *Behavioral Ecology and Sociobiology* **40**, 61–70 (1997).
 22. Vonhelversen, O. & Vonhelversen, D. The Advertisement-Song of the Lesser Noctule Bat (*Nyctalus Leisleri*). *Folia Zoologica* **43**, 331–338 (1994).
 23. Park, K. J., Altringham, J. D. & Jones, G. Assortative roosting in the two phonic types of *Pipistrellus pipistrellus* during the mating season. *Proceedings of the Royal Society B-Biological Sciences* **263**, 1495–1499, <https://doi.org/10.1098/rspb.1996.0218> (1996).
 24. Gerell-Lundberg, K. & Gerell, R. The Mating-Behavior of the Pipistrelle and the Nathusius Pipistrelle (Chiroptera) - a Comparison. *Folia Zoologica* **43**, 315–324 (1994).
 25. Russ, J. & Racey, P. Species-specificity and individual variation in the song of male Nathusius' pipistrelles (*Pipistrellus nathusii*). *Behavioral Ecology and Sociobiology* **61**, 669–677 (2007).
 26. Georgiakakis, P. & Russo, D. The distinctive structure of social calls by Hanák's dwarf bat *Pipistrellus hanaki*. *Acta Chiropterologica* **14**, 167–174 (2012).
 27. Altringham, J. D. & Fenton in *Bat Ecology* (2003).
 28. Russo, D. & Jones, G. The social calls of Kuhl's pipistrelles *Pipistrellus kuhlii* (Kuhl, 1819): structure and variation (Chiroptera: Vespertilionidae). *Journal of Zoology* **249**, 476–481, <https://doi.org/10.1111/j.1469-7998.1999.tb01219.x> (1999).
 29. Racey, P. A. & Swift, S. M. Feeding Ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) During Pregnancy and Lactation. *Journal of Animal Ecology* **54**, 205–215 (1985).
 30. Mcgregor, P. K. Signaling in Territorial Systems - a Context for Individual Identification, Ranging and Eavesdropping. *Philos T Roy Soc B* **340**, 237–244, <https://doi.org/10.1098/rstb.1993.0063> (1993).
 31. Fenton, M. B. Assessing signal variability and reliability: 'to thine ownself be true'. *Animal Behaviour*

- 47, 757–764 (1994).
32. Rydell, J. Feeding Territoriality in Female Northern Bats, *Eptesicus Nilssonii*. *Ethology* **72**, 329–337, <https://doi.org/10.1111/j.1439-0310.1986.tb00633.x> (1986).
 33. Kronwitter, F. *Population structure, habitat use and activity patterns of the noctule bat, Nyctalus noctula Schreb., 1774 (Chiroptera: Vespertilionidae) revealed by radio-tracking.* (1988).
 34. Lewis, T. & Taylor, L. R. Diurnal periodicity of flight by insects. *Transactions of the Royal Entomological Society of London* **116**, 393–435 (1965).
 35. Peng, R. K., Sutton, S. L. & Fletcher, C. R. Spatial and temporal distribution patterns of flying Diptera. *Journal of Zoology* **228**, 329–340, <https://doi.org/10.1111/j.1469-7998.1992.tb04612.x> (1992).
 36. Williams, C. B. Studies in the effect of weather conditions on the activity and abundance of insect populations. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **244**, 331–378 (1961).

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Author contributions

S.G. programmed analysis tools for bearing angle calculation, conducted the audio recordings, extracted the echolocation parameters and reconstructed flight paths. H.U.S., A.D. and S.G. analyzed and interpreted the data, and wrote the manuscript. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Author information and contribution from others to chapter 2

The study was planned by Prof. Dr. Hans-Ulrich Schnitzler, Dr. Annette Denzinger and me, I conducted the audio recordings, extracted the echolocation parameters and reconstructed the flight paths. I analysed and edited the data and wrote the first draft of the manuscript. Prof. Dr. Hans-Ulrich Schnitzler, Dr. Annette Denzinger and I interpreted the data and cooperated in writing the manuscript, which will be submitted soon with Prof. Dr. Hans-Ulrich Schnitzler and Dr. Annette Denzinger as co-authors. Technical resources were provided by Prof. Dr. Hans-Ulrich Schnitzler and Dr. Annette Denzinger.

Pipistrellus pipistrellus foraging in edge space
adjust flight and echolocation behavior
to prevent masking of potential prey

Simone Götze, Annette Denzinger & Hans-Ulrich Schnitzler

Abstract

The echolocation behavior of foraging bats is inseparably connected to the spatial relation between bat, prey, and background. We recorded flight and echolocation behavior of foraging *Pipistrellus pipistrellus* near horizontal and vertical structures around streetlights and in darkness. From multichannel recordings of echolocation signals we reconstructed three-dimensional flight paths of foraging bats and determined how the horizontal distance to streetlamps and the vertical distance to ground affected the average duration of search signals. Foraging bats circled streetlamps mainly at horizontal distances of 2.5 - 9 m. The altitude of search flights differed according to the height of the lamps and the corresponding prey abundance. Many of these flights were found within edge space where bats react to background targets in their echolocation behavior. By using the reduction of the average duration of search signals, we determined the outer horizontal and vertical borders of edge space. We also describe and discuss a yet unknown 'No-Forage-Area' between edge space and background, which was not entered by hunting bats. We conclude that *P. pipistrellus* foraging in edge space adjust their flight and echolocation behavior to prevent the masking of potential prey echoes by background echoes in their search space.

Keywords: edge space, foraging, echolocation behavior, acoustic masking, No-Forage-Area

Introduction

Flight and echolocation are key innovations in the evolution of bats. These two systems have been shaped to cope with the motor and sensory tasks which depend on where bats search for prey, on how they forage, and on what they feed. Highly diverse wing and signal designs found throughout the vast variety of bat species worldwide reflect these adaptations [1, 2]. The spatial relation between bat, target and background structures has a profound impact on flight morphology and echolocation behavior [3-7]. Based on these conditions, bat habitats have been classified into the three types open, edge and narrow space [8]. In open space, bats fly far from background such as vegetation or ground and do not react to background echoes. In edge space, bats forage in the vicinity of background structures and emit echolocation calls, which are adapted for the perceptual separation of target echo and background echo to prevent masking effects. Such call adaptations comprise decreased call durations and pulse intervals and increased bandwidth of emitted signals compared to the species-specific calls emitted in open space. In narrow space, bats forage close to background structures, i.e., within vegetation, where a separation of target and background echo in time is often not possible. Bats that acquire food in narrow space use different foraging strategies to cope with this problem, such as flutter detection, active and passive gleaning [9-12]. When flying in edge and narrow space bats have also to deal with the problem that the background not only produces acoustical clutter echoes but also may be an obstacle which challenges the motor system and must be avoided to prevent collision risk.

Bats using the aerial-hawking echolocation strategy [12] often forage in open space as well as in edge space. The border between edge and open space is indicated by changes in echolocation behavior, when the bats enter their species-specific edge space [13-15]. The border between edge and open space was determined on a rather rough scale for *Pipistrellus pipistrellus* and reported with 3-5 m from background [14, 16].

In this study, we used technically advanced methods to accurately measure the border between open and edge space for *P. pipistrellus*. The echolocation signals of the bats were recorded with an array of 4 microphones while they foraged around a streetlight. This set-up allowed for individual flight path reconstruction and the assignment of measured call parameters to the spatial position and flight direction for each echolocation signal of the emitting bat.

We tested the following hypotheses:

(1) The flight and echolocation behavior of pipistrelles are determined by the distance to background targets.

(2). Foraging bats adjust their flight and echolocation behavior to prevent the masking of prey echoes by background echoes.

(3) The species-specific edge space has an inner and an outer border. The recorded bats oriented in space and searched for food simultaneously, a prerequisite to determine the species-specific horizontal and vertical border between edge and open space.

Our method enabled us to retrace the individual flight and echolocation behavior of foraging bats in relation to background and evaluate their strategies for successful foraging and collision avoidance.

Methods

Species and study sites

Sound recordings of free flying *Pipistrellus pipistrellus* were made from July to October 2010, in May and June 2014 and in August 2015 at four locations in south-western Germany. Two of the recording sites were unlit, located above extended meadows along the skirts of a forest in Einsiedel and along the shore of a lake in Kirchentellinsfurt. These recordings were merged to one dataset and are named 'Darkness' hereinafter. The two other sites were located above meadows and abandoned streets, illuminated by streetlamps at the periphery of residential areas in Bebenhausen and Ofterdingen. Streetlights differed in size, shape, and altitude; the streetlight in Bebenhausen was characterized by an omnidirectional light emitting cylinder of 0.44 m height and a diameter of 0.29 m. The center of the cylinder was mounted on top of a straight, round metal post. The lamp was capped at 3.9 m, limiting most of the illumination to the area around and underneath the light source. The streetlight in Ofterdingen was rather directional with a rectangular light emitting box at an altitude of 7.09 m that was bend to one side, illuminating a sidewalk beside a meadow. The center of the spotlight was beside the bearing metal pole.

Sound recordings

Echolocation signals were recorded by a horizontally oriented T-shaped and planar array consisting of four microphones pointing upwards. Three microphones were positioned in a line and a fourth was fixed at a right angle to the central microphone. All outer microphones

had identical interspaces to the central receiver. Due to technical advancement, we successively used two arrays of different dimensions. The array used in 2010 consisted of four Knowles microphones (Model SPM0404UD5) with interspaces of one meter. In 2014 and 2015 we used a larger array with custom-made microphones fixed at interspaces of two meters to the central microphone.

The arrays were adjusted to heights of 1–1.6 m above ground and positioned >8 m from vertical structures at unlit locations and close to streetlights at illuminated recording sites. Each sound recording was amplified, digitized, and stored as a wav-file using the custom-made software 'Battery'. In 2010, the sound recordings were digitized with a sampling rate of 250 kHz with an A/D-converter of Type USB-6251 (National instruments, Texas). From 2014 to 2015 the sampling rate was increased to 400 kHz and we used an A/D-converter of Type USB-6356 (National instruments, Texas). All recordings were conducted in accordance with relevant guidelines and regulations.

3D flight path reconstruction and angulation

Echolocation signals emitted by bats within the recording range of the T-shaped array were recorded at different arrival times due to different distances between the bat and the microphones. These delays were calculated by measuring the time lag at which the cross-correlation function between the signal of the reference microphone and the signal of each of the other three microphones reached the maximum value. The three calculated differences in arrival time allowed a conversion into three-dimensional spatial positions of the bat in the moment of signal emission relative to the array. Spatial positions in chronological order revealed individual flight paths of the bats. The actual altitude of the bats above ground was calculated for every echolocation call in respect to the surrounding landscape. Above even ground, the height at which the array was mounted added to the reconstructed flight level above the array to determine the actual altitude of the bats. One of the streetlights was positioned on top on a shelving meadow, here we measured and reconstructed the slope of the ground and calculated the vertical distance for every signal depending on the three-dimensional position around the streetlamp.

Plane angles and distances between bats and streetlights were calculated for all distinguished spatial positions at search call emissions in flight below and at the height of the considered streetlight. For each search call, an angle was calculated between a 'flight

direction vector' and an 'obstacle vector'. The flight direction vector was defined by the spatial positions of the bat in the moment of search call emission and of the preceding search call emission unless their pulse interval exceeded 200 ms. Successive signals with pulse intervals of more than 200 ms were excluded from angulation. The obstacle vector was composed of the spatial position of the bat in the moment of search call emission and the position of the streetlight at the bat's altitude.

Data set and analysis

To distinguish the border between open and edge space we present signal parameters and corresponding flight positions of 1523 echolocation signals. Only echolocation calls of single flying pipistrelles with pulse intervals of > 63 ms were considered to exclude acoustic reactions in encounters with other bats as well as approach calls and feeding buzzes emitted before insect catches. Furthermore, we controlled for vertical effects on the determination of the horizontal border between edge and open space and for horizontal effects on the determination of the vertical border. For this reason, we distinguished the altitude of the vertical border between open and edge space first at unlit sites, where vertical structures were at least 8 m away. With the identified value acting as a reference point for signals to be excluded for the determination of horizontal effects, we used only signals that were emitted above an altitude of > 5.8 m for the determination of the horizontal border between open and edge space. With the value of the horizontal border, we excluded signals within a zone of 15 m in diameter around the streetlamps for the measurement of the vertical border at illuminated sites.

The minimum distance between foraging pipistrelles and background was determined by reconstructed flight paths of bats flying alone or with conspecifics comprising 8190 search and approach calls. The minimum distance to vertical structures such as streetlamp posts and light emitting cylinders was evaluated by signals which were emitted at altitudes below or at the maximum heights of the relevant streetlights.

Recorded signals were visualized as color spectrograms (FFT 512, Blackman window, dynamic range of 90 dB) using custom-made software (Selena, University of Tuebingen, Germany). The spectrograms were plotted with a temporal resolution of 0.06 ms and a spectral resolution of 117.5 Hz due to auto-padding and time interpolation. The beginning and end of signals were measured in the spectrograms using the automatically applied

criterion of – 15 dB below highest amplitude. The signal parameters duration, pulse interval, peak frequency, and terminal frequency were measured, with focus on the parameter signal duration as will be found in the results section. According to the criterion, that bats react to background in their echolocation behavior in edge space, but do not change their signal parameters in open space, we applied a piecewise (2-segment) linear regression breakpoint analysis to reveal changes in signal parameters in relation to horizontal and vertical distance to background to distinguish the border between edge and open space. Additionally, call duration was averaged within bins of 1 m horizontal and vertical distance to background, respectively.

Results

The flight and echolocation behavior of foraging common pipistrelles (*Pipistrellus pipistrellus*) was recorded in darkness and at two different locations close to streetlights. The reconstruction of the bat's flight paths allowed for a three-dimensional representation of their distribution in space throughout several hours of nocturnal activity. Pipistrelles foraging at naturally dark sites showed an evenly and omnidirectional distribution of spatial positions identified by emitted search calls in flight (n=3323, Figure 1, 'Darkness'). In presence of streetlights, bats followed rather circular, repetitive flight paths beside or around the lamp structures (n=3122 (Bebenhausen), n=2665 (Ofterdingen), Figure 1). Approach calls were embedded additionally as red data points in all figures depicting the spatial distribution of foraging bats for means of completeness.

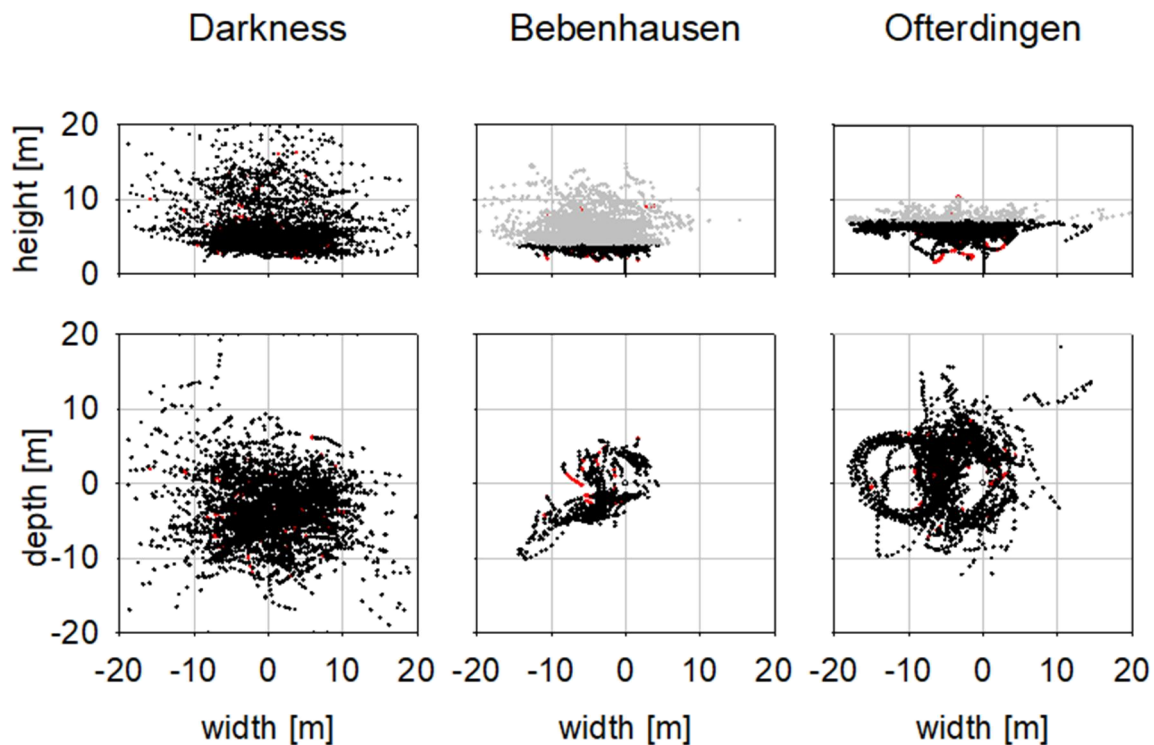


Fig.1: Reconstructed flight paths of foraging *Pipistrellus pipistrellus* in darkness and around streetlights. Flight path reconstructions accumulated over time and shown from lateral view (top panels) and from top view (bottom panels). Search calls (black dots) and approach calls (red dots) are depicted above the central microphone of the recording device in Darkness and around streetlights (black vertical lines in lateral view panels and black o in top view panels). Search calls emitted above streetlight altitude (grey dots) were excluded in the bottom panels to give view to the distribution of flight paths around the vertical streetlight structures.

Vertical border between open and edge space of Pipistrellus pipistrellus

The vertical border between open and edge space was determined at unlit and illuminated foraging sites by parameter changes in echolocation signals of *Pipistrellus pipistrellus*. According to significant reduction in search call duration the border was found at 4.36 ± 0.19 m (mean \pm SEM, $n=160$, Sigmaplot 10.0) above ground at unlit sites. At illuminated sites the bats reacted to ground below 4.59 ± 0.31 m (mean \pm SEM, $n=197$, Sigmaplot 10.0) in Bebenhausen and below 5.2 ± 0.23 m (mean \pm SEM, $n=320$, Sigmaplot 10.0) in Offerdingen. Data bins of 1 m revealed decreasing call duration with decreasing distance to ground within edge space in the vertical (Fig.2).

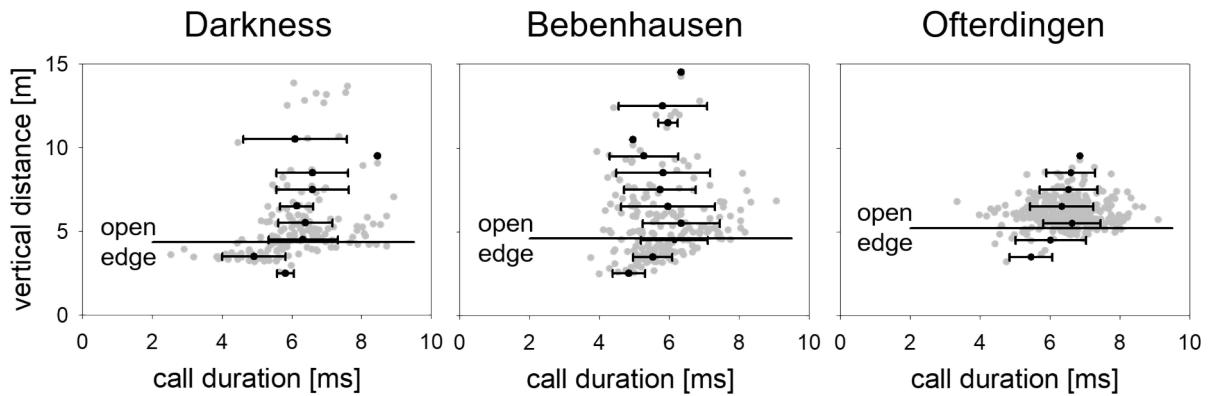


Fig 2: Vertical border between open and edge space for *Pipistrellus pipistrellus*. Determination of significant reduction in average call duration of search calls at unlit (Darkness) and illuminated foraging sites (Bebenhausen, Ofterdingen) revealed the border between open and edge space (black lines). Means and standard deviations of call duration are calculated for data bins of 1 m vertical distance to ground and indicate decreasing call duration within edge space.

*Horizontal border between open and edge space of *Pipistrellus pipistrellus**

The horizontal border between edge and open space was determined by changes in echolocation signal structure around the streetlamp pole in Ofterdingen. The streetlight in Bebenhausen only scaled up to 3.9 m and signals emitted around the structure may have been affected by the vertical effect of background. Since all echolocation calls below 5.8 m were excluded for the separation from vertical effects, the site was unsuitable for the measurement of horizontal effects. At the unlit sites, an even vertical structure was lacking and prevented a determination of the horizontal border between open and edge space. In Ofterdingen, breakpoint analysis of call duration in search flight revealed reactions in echolocation behavior to the vertical streetlamp structure beyond horizontal distances of $4.73 \text{ m} \pm 0.23 \text{ m}$ (mean \pm SEM, $n=272$, Sigmaplot 10.0). Data bins of 1 m distance to lamp structure revealed decreasing call duration with decreasing distance within edge space (Fig.3).

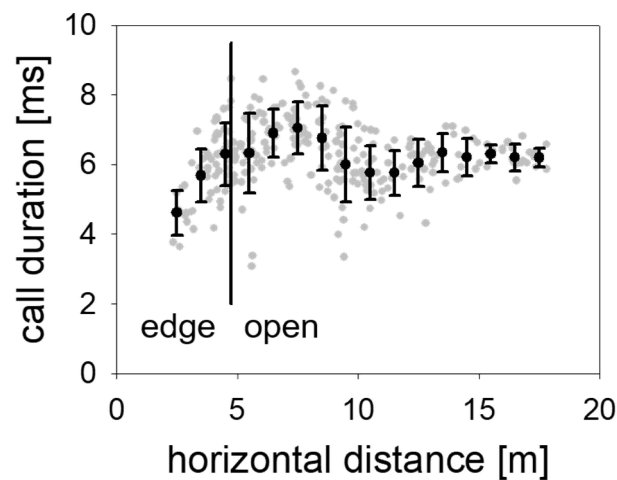


Fig 3: Horizontal border between open and edge space for *Pipistrellus pipistrellus*. Determination of significant average reduction of call duration at the illuminated foraging site Ofterdingen revealed the horizontal border between open and edge space (black line). Means and standard deviations of call duration are calculated for data bins of 1 m horizontal distance to streetlamp structure and indicate decreasing call duration within edge space.

Horizontal and vertical No-Forage-Area

The reconstruction of flightpaths around streetlamps and in darkness comprising search and approach calls of foraging pipistrelles revealed a minimum distance between the bats and background structures (Fig. 1, 4). Pipistrelles were not traced below 1.52 m above ground in darkness (n=2109), and below 1.66 m (Bebenhausen, n=3293) and 1.54 m (Ofterdingen, n=2788) at illuminated sites. We did not observe any ruptures in flight paths passing these borders downward. Instead, the located bat positions reflected an ascent in altitude before reaching the lower limit of flight space. The microphone arrays were positioned at lower levels and bats could have been located at minor altitudes. The reconstruction of flightpaths around streetlamps uncovered a minimum distance to vertical structures of 1.03 m at both locations in Bebenhausen and Ofterdingen. Pipistrelles were not traced within this No-Forage-Area at all altitudes around the vertical structures (Fig. 4, Fig.5). The same was the case for recent set up microphone arrays, where the bats seemed to survey the foreign structure, but kept a minimum distance of at least 1.04 m.

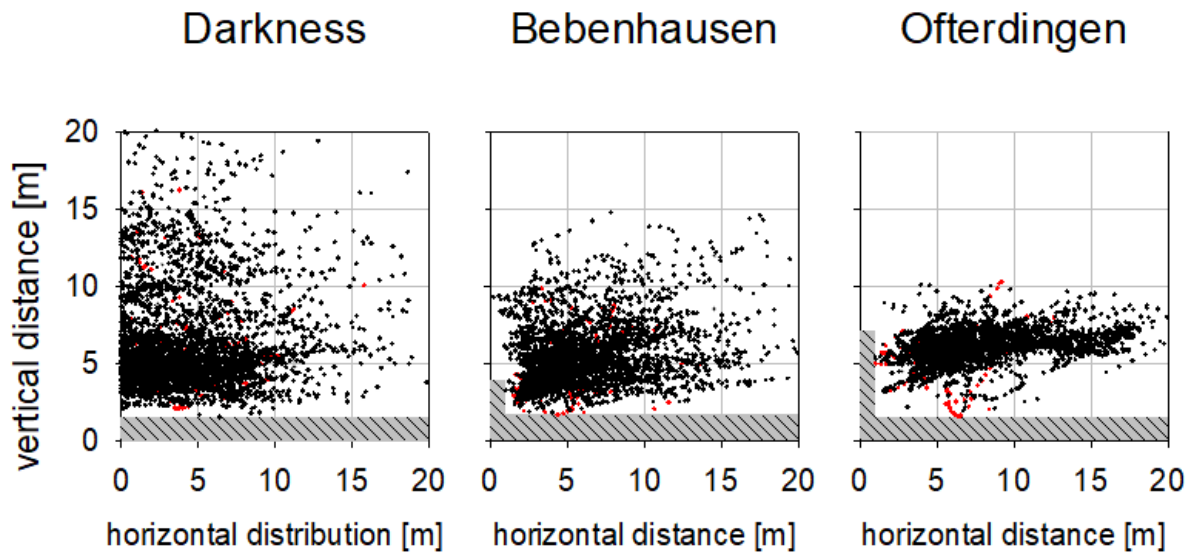


Fig. 4: Horizontal and vertical No-Forage-Area of *Pipistrellus pipistrellus*. Spatial positions of bats during search (black dots) and approach (red dots) call emission are shown by their vertical distances to ground at all recording sites and horizontal distances to streetlamp structures in Bebenhausen and Offerdingen. No-Forage-areas are shaded (grey). Depicted horizontal No-Forage-Areas correspond to correlating lamp heights.

Flight path orientation around streetlights

The reconstruction of search flights of foraging pipistrelles near streetlights revealed many circular paths around the lamp structures which individual bats followed in a repetitive manner (Fig. 5). The No-Forage-Areas were not entered at any time.

Measurements of distances and angles between the foraging bats and streetlight structures revealed a minor flight activity within a radius of 3 m around the streetlights and major flight activity at distances of 4-6 m (Figure 6). A percentage of 10 % (n=531) of 4931 echolocation calls emitted within 10 m horizontal radius around the two light sources was measured at distances between 1.03 and 3 m. 30 % of the signals (n=1533) were emitted between 3 and 5 m horizontal distance, which is mostly within the species-specific edge space. The most frequently chosen distance of foraging bats to streetlights was found between 4 and 6 m, where 35.5 % (n=1756) of the echolocation signals were emitted in foraging flight.

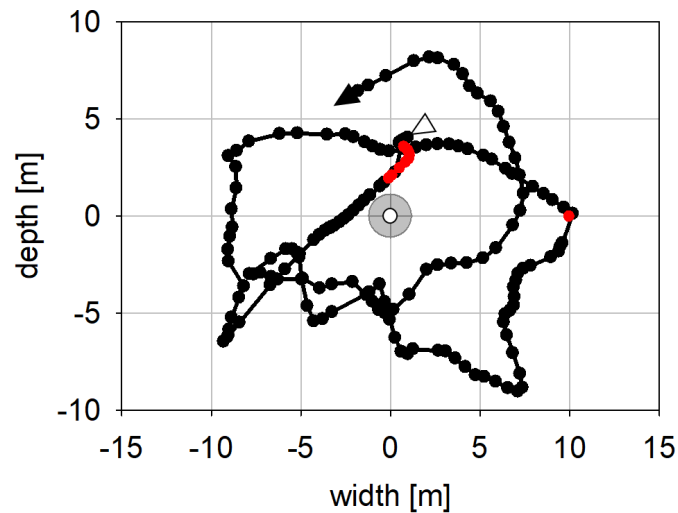


Fig. 5: Reconstructed exemplary flight path of a foraging *Pipistrellus pipistrellus*. Successive search calls (black dots) form a flightpath (black line) around the streetlamp (central black o) and associated No-Forage-Area (circular grey zone, shown from top view). The beginning of the exemplary flight path is marked by an open triangle, the end is marked by a filled triangle.

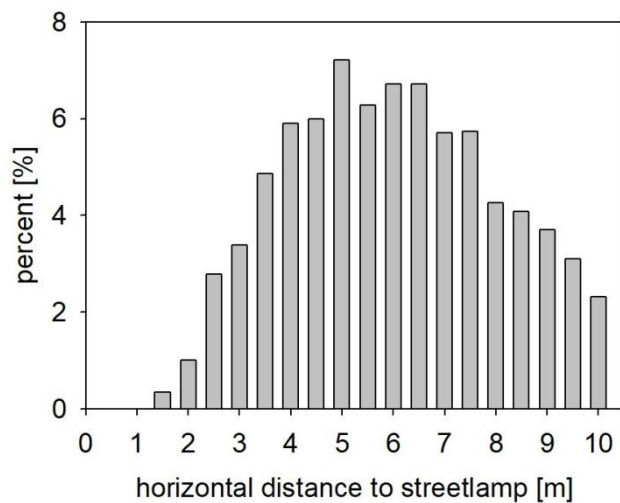


Fig. 6: Spatial distribution of pipistrelles search calls around streetlights. Number of emitted search calls within 10 m radius around streetlights grouped in bins of 0.5 m horizontal distance.

At distances beyond 4.73 m from the streetlights, which is the identified species-specific border between edge and open space, the pipistrelles flew in open space and did not react to the streetlight with a reduction of the average call duration. Flight paths within edge

space were oriented tangentially around the streetlight, indicated by average angles of ~ 90 degrees between bats and lamp structure. Pipistrelles flying at distances below 1.85 m to the streetlamp structure kept a minimum angle of 57 degrees (Fig. 7).

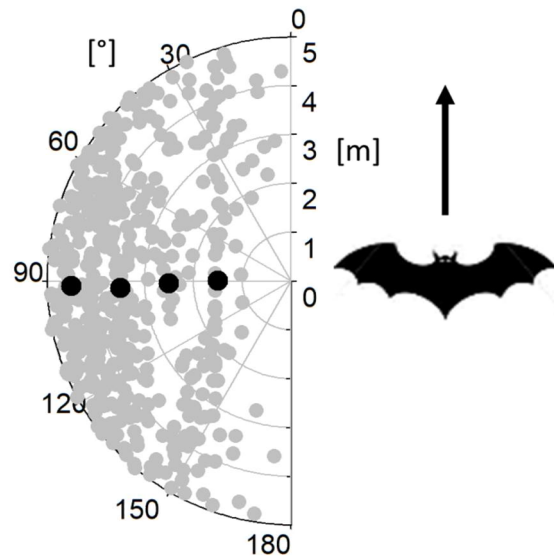


Fig. 7: Angles and distances between foraging pipistrelles and streetlamps.

Angles between the flight direction vector of bats and the lamp structure at corresponding distances (grey dots) were distinguished for successive search calls of individual bats. Means are included as black dots.

Impact of streetlights on flight altitude

A comparison of reconstructed flightpaths incorporating all echolocation calls in single flight showed significant differences in the spatial distribution of pipistrelles altitudes according to recording site. At dark sites, without interference by artificial light sources, pipistrelles foraged at altitudes of 5.84 m (Median, Q1: 4.52 m, Q3: 9.17 m, $n=776$). Around an insect-attracting streetlight at a height of 7.09 m (Ofterdingen), the flight altitude significantly increased to 6.06 m (Median, Q1: 5.41 m, Q3: 6.66 m, $n=2788$), whereas at the considerably lower artificial light source at 3.9 m in Bebenhausen the foraging pipistrelles decreased their altitudes to 5.02 m (Median, Q1: 4.03 m, Q3: 6.44 m, $n=3293$, Fig. 8).

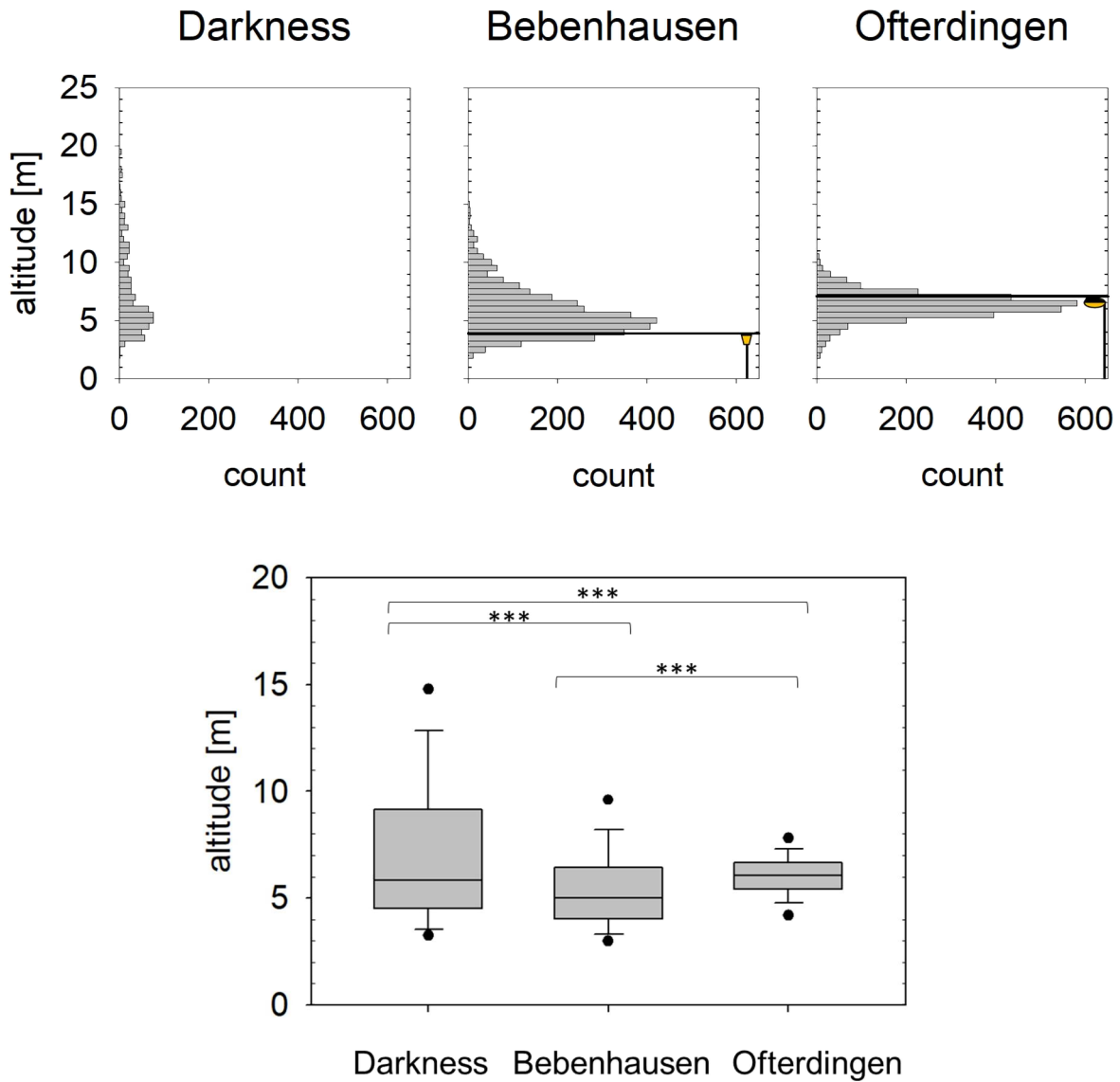


Fig.8: Flight altitudes of *Pipistrellus pipistrellus* at three different foraging sites. Heights of streetlights are depicted in the histograms (black horizontal lines). Distributions of foraging pipistrelles differ significantly in altitude (Boxplot).

Discussion

Bat habitats have been classified into the three habitat types open, edge and narrow space according to the spatial relations between a bat, its prey, and background structures [4, 8]. In this study we investigated the echolocation and flight behavior of *Pipistrellus pipistrellus* foraging in open and in edge space near streetlights. Streetlights are advantageous for light-tolerant bat species because they attract prey insects by their light emission and thus offer an area with beneficial foraging conditions. However, streetlights are also potential obstacles which must be avoided, and they produce clutter echoes which could mask the echoes of potential prey. When a bat forages near a streetlight, echolocation has two functions at the same time. It allows the bat to determine its

position in relation to the lamp and it enables the bat to find and localize prey. We hypothesized that the echolocation and flight behavior of the bats are determined by the distance to background and are adjusted to reduce the risk of masking of prey echoes by clutter echoes and to prevent collisions with the lamp post. We further hypothesized that the species-specific edge space is not only characterized by an outer border, but also by an inner border towards background.

Border between edge and open space

Bats flying in open space are far from background and obstacles and do not react to such targets. Their echolocation is mainly used to find prey. In edge space, bats react to background targets and emit signals that are adapted to deal with the perceptual tasks of detection, localization, and characterization of prey in the presence of background echoes and to be used also for orientation in space and obstacle avoidance. The distance to background plays an important role in this process which is indicated by a reduction of the mean values of call duration and pulse interval and an increase of the mean bandwidth with decreasing distance to background [4, 13, 15, 17, 18].

The echolocation behavior of *Pipistrellus pipistrellus* foraging in uncluttered open and in background cluttered edge situations has been described in the following way. When foraging in open space *P. pipistrellus* used quasi-constant frequency (QCF) calls with durations of 4-9 ms (mean=6.2 ms) and mean bandwidths of 7 kHz. The signals were usually emitted at regular pulse intervals of ~97 ms or sometimes with call omissions at intervals of 180 or 270 ms. When flying in edge space *P. pipistrellus* emitted frequency-modulated (FM) signals with shorter durations of 3-6 ms and mean bandwidths of 31 kHz at decreased mean pulse intervals of 85 ms [14, 19]. The beginning of distant-dependent changes of call parameters is an unambiguous indicator for the bat crossing the border from open to edge space and has been used to determine the outer border of edge space for *Vespertilio murinus* in transfer flight above flat ground along a building [13]. For several other species such as *Pipistrellus kuhlii*, *P. pipistrellus*, *P. nathusii* [14] and *Eptesicus serotinus* [15] the outer border of edge space was also either measured or estimated visually while the bats were foraging near extended vertical structures such as vegetation edges, along buildings and/or above the ground. The width of edge space hereby seems to be species-specific. Widths of 8-10 m in *Eptesicus serotinus* [15] and of 5-6 m in *Vespertilio murinus* (Schaub and Schnitzler 2007) have been estimated. A width of 3-5 m was reported for *P. pipistrellus* [14].

Foraging bats must prevent masking of echoes from targets of interest. Forward masking occurs if the prey is positioned within the signal overlap zone, where prey echoes are masked by the emitted echolocation signal. When prey is positioned within the clutter overlap zone, the prey echoes are masked by echoes of background structures. No masking occurs in the overlap free window between signal and clutter overlap zone. The bats' edge space reactions to background structures have the

function to reduce the risk of masking. A decrease of signal duration reduces the width of the signal and clutter overlap zone thus increasing the width of the overlap free window between the two zones [14]. The width of each zone is determined by the call duration and increases by 0.17 m per ms. Thus, echolocation signals emitted in open space with an average duration of 4-9 ms result in signal overlap zones of 0.68-1.53 m. Signals with call durations of 3-6 ms, which are typically emitted in edge space, lead to signal overlap zones of 0.51-1.02 m and clutter overlap zones of the same extent.

We determined the species-specific horizontal and vertical outer border of edge space in the common pipistrelle bat (*P. pipistrellus*) by evaluating changes of the call parameter duration in search signals in relation to the horizontal distance to streetlights and the vertical distance to the ground. The vertical border was similar across all recording sites (4.36 m in darkness, 4.59 m and 5.2 m above ground around streetlights), confirming previous results on a more precise scale due to advanced technical methods.

The horizontal border between edge and open space could be measured only at one recording site, due to lacking vertical structures at dark recording sites and exclusion of data points to avoid vertical effects at the streetlight in Bebenhausen. The horizontal border did not differ substantially from the border in the vertical (horizontal border: 4.73 m, vertical border: 5.2 m). We will discuss the flight and echolocation behavior of foraging bats around the vertical streetlamp posts at the two illuminated recording sites and above ground separately hereafter.

Flight and echolocation behavior around streetlights

We confirmed that *Pipistrellus pipistrellus* often foraged around streetlights for airborne prey [20, 21] thereby benefitting from the attracting character of artificial light sources for insects such as Diptera, Chironomids and Trichoptera [22], which accumulate around streetlights [21] and represent a large portion of pipistrelles diet. When searching for prey pipistrelles often make scanning movements with their head and actively move their rather directed sonar beam in horizontal and vertical direction which results in a widening of their search cone. Scanning behavior increases the search angle of the pipistrelles' search cone from 120 - 150° to up to 180° and thus improves the chance for prey detection [18, 19, 23].

The search space, i.e., the spatial volume in the search cone where bats most likely expect, search for, and detect prey is restricted in its angular extent by the search cone angle which is determined by the width of the sonar beam and the degree of head movement in scanning behavior. The search space is also limited in length due to the range in which insects can be detected. Detection distances of 1.4 - 2.1 m (mean=1.7 m) have been measured for pipistrelle bats foraging near streetlamps [24]. The minimum detection distance is set by the extent of the signal overlap zone, which depends on

the chosen call duration [17]. Correspondingly, the search space of pipistrelles foraging near streetlamps would have an angular extent of up to 180° and a depth from the end of the signal overlap zone to about the longest observed detection distance at 2.1 m. Within this limited section of the search cone the foraging pipistrelles expect prey. It may well be that the detection distance may be longer in open space, either associated with increased sound pressure level of the echolocation calls or with larger prey such as moths [18, 19]. In any case, bats foraging in edge space must keep their search space free of masking to be successful.

In a first approximation an insect echo is masked if the insect is positioned in the signal or in the clutter overlap zone and can be detected if it is positioned in the overlap free window between the two zones. Masking effects are additionally reduced by spatial unmasking with an increasing angle between the incoming insect echo and clutter echoes [25]. A good strategy for masking prevention is therefore to keep the overlap free window larger than the search space width and to choose flight paths which result in a distinct angular separation of insect and clutter echoes. In our study the foraging bats circled around the streetlamps and emitted search signals during foraging flights mainly at distances of 2.5-9 m to the lamps. Shorter distances below 2.5 m were rare. In a scenario which describes the flight and echolocation behavior of bats foraging in open space which means at distances to the lamp post of 4.73 m and more and at signal durations of about 6 ms the clutter overlap zone of lamp echoes begins at 3.71 m or more and the signal overlap zone ends at 1.02 m relative to the bat. The resulting overlap free window reaches from 1.02-3.71 m or more and is wide enough for a search space which is estimated to extend from 1.4 to 2.1 m. We assume that this is the reason why *P. pipistrellus* did not react to the background when foraging in open space beyond 4.73 m to the lamp post.

In a scenario where a bat circles the lamp at 2.5 m with a signal lasting 3 ms the clutter overlap zone is reduced to 0.51 m due to the shorter signal duration and begins at 1.99 m from the bat. The signal overlap zone ends at 0.51 m so that the overlap free window extends from 0.51-1.99 m, still covering the estimated search space with a mean detection distance of 1.7 m. Possible masking effects by clutter echoes are further reduced due to spatial unmasking, as the bats flew in more or less circular flight paths around the streetlights which resulted in large clutter echo angles.

Additionally, they increase the bandwidth and with it the sweep rate of their FM signals. This improves the ability of the bats to determine the distance to background targets and to characterize their nature. Additionally, a higher sweep rate has effect that the signals activate the tuning curve of auditory neurons for a shorter time than QCF signals which reduces masking. Such an increase in bandwidth in edge situations has been described by Kalko and Schnitzler (1993) and was also observed by us in relation to the lamp but not quantified. The strongest effect on the width of the overlap free window has the distance of the pathways to the lamp post. We assume that this is the

reason why most search signals were emitted at distances of more than 2.5 m. Only in a very few cases bats came closer to the lamp and thereby emitted signals with a mean duration of about 2.6 ms at 2 m to the lamp. In such a scenario signal and clutter overlap zone are each 0.44 m wide and the overlap free window zone extends therefore only from 0.44 to 1.56 m. Even if a strong angular unmasking is considered the chances to detect insects are highly reduced. This may be the reason why *P. pipistrellus* only rarely flew at distances of less than 2.5 m to the lamp post.

Remarkably, the flightpaths reconstructed from search and approach signals of foraging pipistrelles revealed an area of constant width around streetlights that was not entered by the bats at any time. The extent of this horizontal 'No-Forage-Area' was measured with 1.03 m around both streetlights. This area may be established by two factors. One may be a sensory limit for echolocation to find prey flying close to background. Another limitation may depend on the motor capabilities of a species which determine the required motor space to catch prey near background to avoid collisions with it. At signal durations of 3 ms, both signal and clutter overlap zone measure 0.51 m and the overlap free window is closed if the distance between bat and background is below 1.02 m. Within a lesser distance to background the bat could no longer receive target echoes without overlap. Interestingly, the measured width of the No-Forage-Area of 1.03 m determined from search calls corresponds to a scene where minimal call duration of 3 ms results in the closing of the overlap free window. This correlation suggests that *P. pipistrellus* avoids areas where the overlap free window is closed even if angular spatial unmasking would make it possible to still detect prey insects at lateral positions to the flight path. A possible reason for that may be the high amplitude of the streetlamp echo, which could suspend the beneficial effects of spatial unmasking.

The motor task to catch insects near the lamp and to avoid collisions with it gets more difficult with decreasing distance to background. Motor space is correlated with size-dependent characteristics such as weight, wing loading, aspect ratio, wing shape and the resulting flight speed range [21, 26]. *P. pipistrellus* is a rather fast flying bat with speed of 5.7 -7.3 m/s in open space, but is also able to forage in edge space at lower speeds of 1.5 - 3.5 m/s [18]. In an earlier study, we documented a flight of a residential pipistrelle chasing an intruder off its occupied foraging area around a streetlight, where the bat passed the lamp structure at 0.68 m. This singular example supports the assumption that pipistrelles can fly closer to background than indicated by the width of the No-Forage-Area if they do not forage. However, in this situation the bat emitted very short signals for the purpose of obstacle avoidance in flight [27, 28]. These results suggest that the No-Forage-Area is determined by sensory limitations rather than motor limitations.

Although insect prey abundance is highest at the light source, we show here why successful foraging is only possible if the bats keep a certain distance to the lamp. The bats circled around the lamps mainly at distances beyond 2.5 m and chose signal durations so that the overlap free window was

wide enough to cover the species-specific search space. The echolocation and flight data suggest that bats foraging in edge space chose a behavioral strategy which prevented the risk of masking of insect echoes in their search space.

Flight and echolocation behavior in relation to ground

The flight situation of foraging bats above flat ground without vertical structures is different from foraging around streetlights. In contrast to vertical streetlights the ground does not attract prey insects and the collision risk for bats is lower since their flights are mostly directed horizontally. Without the impact of artificial light, pipistrelles foraged for airborne prey within a widespread vertical distribution reaching from 2 - 20 m above ground at an average height of 5.8 m. Most search flights were found between 4.5-9 m. The echolocation behavior indicated a vertical border between open and edge space at 4.36 m. Foraging flights below 3m were rare.

The foraging flight altitude at the streetlights was influenced by the height of the relevant lamp. At Bebenhausen, at a lamp height of 3.9 m, most search flights were found between 4 -6.5m at an average altitude of 5 m. The upper edge space border was measured at 4.59 m and foraging flights below 3 m were rare. In Ofterdingen, at a remarkably higher lamp height of 7.09 m, most search flights were found at a significantly higher average altitude of 6.06 m between 5.4 - 6.7 m. The upper edge space border was found at 5.2 m and flights below 4.2 m were rare.

The vertical border of edge space was similar across all recording sites and similar to the horizontal border of 4.73 m. That suggests that alone the distance to background determines the beginning of edge space. It even plays no role whether there is light or no light and whether the clutter producing background target is an isolated lamp post which forms an edge space island in horizontal direction or an extended structure as the flat ground below the lamps and at the dark recording site in vertical direction. In horizontal as well as in vertical edge space situations the bats chose a behavioral strategy which prevented the risk of masking of insect echoes in their search space and searched for prey mainly at distances of more than 2.5 m to the clutter producing background. Furthermore, it seems to make no difference whether the masking clutter comes from the side as from the lamp or from below as from the ground. The observation that pipistrelles fly along extended vertical structures with a distance of at least 2 m [14] also suggests that alone the distance to clutter determines edge space and that pipistrelles keep a certain distance to background to avoid masking. Foraging bats adjusted the height of their search flights according to the occurrences of insects to improve their insect catch success rate. The wide vertical distribution in aerosphere at dark foraging sites accounts for that in the same way as the fact that flight altitudes changed according to light distribution and the corresponding prey abundance at streetlights.

P. pipistrellus was never traced at flight heights below 1.66 resp. 1.54 m at the two lamp sides. We see no arguments why this vertical No-Forage-Area should be larger than the horizontal No-Forage-Area described above, and we assume that neither sensory nor motor limitations explain this result. Most likely the bats did not enter this area as there were not enough prey insects flying near the ground.

We conclude for pipistrelles and most likely also for other edge space foragers that the width of the horizontal and vertical edge spaces and the widths of the No-Forage-Areas are species-specific and determined by signal design and associated sensory limitations. Apart from these given conditions which reflect the bats' attitude to avoid masking they chose their preferred flight altitude according to food abundance to enhance their foraging success.

1. Denzinger, A., et al., Guild Structure and Niche Differentiation in Echolocating Bats, in Bat Bioacoustics, M.B. Fenton, et al., Editors. 2016, Springer: New York.
2. Denzinger, A. and H.U. Schnitzler, Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Front Physiol*, 2013. 4: p. 164.
3. Fenton, M.B., The foraging behaviour and ecology of animal-eating bats. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 1990. 68(3): p. 411-422.
4. Schnitzler, H.U. and E.K.V. Kalko, Echolocation by insect-eating bats. *Bioscience*, 2001. 51(7): p. 557-569.
5. Schnitzler, H.U. and E.K.V. Kalko, How Echolocating Bats Search And Find Food, in *Bat Biology And Conservation*, T.H. Kunz and P.A. Racey, Editors. 1998, Smithsonian Institution Press: Washington. p. 183-196.
6. Aldridge, H. and I. Rautenbach, Morphology, echolocation and resource partitioning in insectivorous bats. *The Journal of Animal Ecology*, 1987: p. 763-778.
7. Neuweiler, G., Foraging ecology and audition in echolocating bats. *Trends in ecology & evolution*, 1989. 4(6): p. 160-166.
8. Schnitzler, H.-U., C.F. Moss, and A. Denzinger, From spatial orientation to food acquisition in echolocating bats. *Trends in Ecology & Evolution*, 2003. 18(8): p. 386-394.
9. Schnitzler, H.-U., Control of Doppler shift compensation in the greater horseshoe bat, *Rhinolophus ferrumequinum*. *Journal of comparative physiology*, 1973. 82(1): p. 79-92.
10. Schoeppler, D., H.-U. Schnitzler, and A. Denzinger, Precise Doppler shift compensation in the hipposiderid bat, *Hipposideros armiger*. *Scientific reports*, 2018. 8(1): p. 1-11.
11. Denzinger, A. and H. Schnitzler, Perceptual tasks in echolocating bats. *Dynamic perception*, 2004: p. 33-38.

12. Denzinger, A., M. Tschapka, and H.-U. Schnitzler, The role of echolocation strategies for niche differentiation in bats. *Canadian Journal of Zoology*, 2018. 96(3): p. 171-181.
13. Schaub, A. and H.U. Schnitzler, Echolocation behavior of the bat *Vespertilio murinus* reveals the border between the habitat types “edge” and “open space”. *Behavioral Ecology and Sociobiology*, 2006. 61(4): p. 513-523.
14. Kalko, E.K.V. and H.U. Schnitzler, Plasticity in echolocation signals of European pipistrelle bats in search flight: implications for habitat use and prey detection. *Behavioral Ecology and Sociobiology*, 1993. 33(6): p. 415-428.
15. Jensen, M.E. and L.A. Miller, Echolocation signals of the bat *Eptesicus serotinus* recorded using a vertical microphone array: effect of flight altitude on searching signals. *Behavioral Ecology and Sociobiology*, 1999. 47(1): p. 60-69.
16. Schaub, A. and H.U. Schnitzler, Flight and echolocation behaviour of three vespertilionid bat species while commuting on flyways. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, 2007. 193(12): p. 1185-94.
17. Schnitzler, H.-U., et al., The echolocation and hunting behavior of the bat, *Pipistrellus kuhli*. *Journal of Comparative Physiology A*, 1987. 161(2): p. 267-274.
18. Kalko, E.K.V., Insect pursuit, prey capture and echolocation in pipistrelle bats (Microchiroptera). *Animal Behaviour*, 1995. 50(4): p. 861-880.
19. Seibert, A.M., et al., Scanning behavior in echolocating common pipistrelle bats (*Pipistrellus pipistrellus*). *PLoS One*, 2013. 8(4): p. e60752.
20. Haffner, M. and H. Stutz, Abundance of *Pipistrellus pipistrellus* and *Pipistrellus kuhlii* foraging at street-lamps. *Myotis*, 1985. 23(24): p. 167-172.
21. Rydell, J., Exploitation of Insects around Streetlamps by Bats in Sweden. *Functional Ecology*, 1992. 6(6): p. 744-750.
22. Racey, P.A. and S.M. Swift, Feeding Ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) During Pregnancy and Lactation. *Journal of Animal Ecology*, 1985. 54(1): p. 205-215.
23. Kalko, E., Das Echoortungs- und Jagdverhalten der drei europäischen Zwergfledermausarten, *Pipistrellus pipistrellus* (Schreber 1774), *Pipistrellus nathusii* (Keyserling et Blasius 1939) und *Pipistrellus kuhli* (Kuhl 1819), im Freiland. . 1991, University of Tübingen. p. 1-310.
24. Kalko, E.K. Echolocation signal design, foraging habitats and guild structure in six Neotropical sheath-tailed bats (Emballonuridae). in *Symposia of the Zoological Society of London*. 1995. London: The Society, 1960-1999.
25. Sümer, S., A. Denzinger, and H.-U. Schnitzler, Spatial unmasking in the echolocating Big Brown Bat, *Eptesicus fuscus*. *Journal of Comparative Physiology A*, 2009. 195(5): p. 463-472.
26. Aldridge, H., Turning flight of bats. *Journal of Experimental Biology*, 1987. 128(1): p. 419-425.

27. Götze, S., et al., No evidence for spectral jamming avoidance in echolocation behavior of foraging pipistrelle bats. *Sci Rep*, 2016. 6: p. 30978.
28. Götze, S., A. Denzinger, and H.-U. Schnitzler, High frequency social calls indicate food source defense in foraging Common pipistrelle bats. *Scientific reports*, 2020. 10(1): p. 1-9.

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