Coding of spatial and temporal frequency in bat biosonar

Dissertation
an der Fakultät für Biologie
der Ludwig-Maximilians-Universität
München

Anne Leonie Baier

München, 9. Oktober 2018

Coding of spatial and temporal frequency in bat biosonar

Dissertation
an der Fakultät für Biologie
der Ludwig-Maximilians-Universität
München

Anne Leonie Baier

München, 9. Oktober 2018

Erstgutachter: Prof. Dr. Lutz Wiegrebe
Zweitgutachterin: Prof. Dr. Laura Busse
Tag der Abgabe: 9. Oktober 2018
Tag der mündlichen Prüfung: 25. Januar 2019

EIDESSTATTLICHE VERSICHERUNG UND ERKLÄRUNG

Hiermit versichere ich an Eides statt, dass ich die Dissertation „Coding of spatial and temporal frequency in bat biosonar" selbstständig und ohne unerlaubte fremde Hilfe angefertigt habe und keine anderen als die von mir angegebenen Schriften und Hilfsmittel benutzt wurden. Die den benutzten Werken wörtlich und inhaltlich entnommenen Stellen sind kenntlich gemacht.

München, den 9.10.18

Leonie Baier

Hiermit erkläre ich, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist. Im Weiteren erkläre ich, dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe oder ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich einer Doktorprüfung zu unterziehen.

München, den 9.10.18

Leonie Baier

CONTENT

Abbreviations

FM	frequency modulating / frequency modulation
CF	constant frequency
2AFC	two-alternative forced choice
JND	just noticeable difference
ICI	inter-call interval
AM	amplitude modulation
SAM	sinusoidal amplitude modulation

ZUSAMMENFASSUNG

Fledermäuse senden Ultraschallrufe aus und lauschen auf Echos um sich in ihrer Umgebung zu orientieren und Beute zu jagen. Dank dieser Fähigkeit zur Echoortung sowie zum aktiven Flug haben sich Fledermäuse eine überaus ergiebige ökologische Nische erschlossen, den nächtlichen Luftraum. Ihr "sechster Sinn" hat Fledermäusen also Unabhängigkeit vom Sonnenlicht beschert. Aber inwiefern kann Hören Sehen ersetzen? Die vorliegende Arbeit beschäftigt sich mit der Frage wie Echoortung bestimmte räumliche und zeitliche Parameter der Umgebung verarbeitet.

Wenn es um die Wahrnehmung räumlicher Strukturen geht, stehen echoortende Tiere vor einer speziellen Herausforderung. Die Cochlea, das Sinnesepithel des Hörsystems, kann Rauminformation nicht direkt kodieren. Stattdessen muss Rauminformation errechnet werden, über den Vergleich der Signale an beiden Ohren. Im ersten Kapitel dieser Dissertation teste ich die Hypothese, dass Echoortung dennoch Raumfrequenzen heranzieht um ein Bild der Umgebung zu formen. Das Konzept der Raumfrequenz spielte eine entscheidende Rolle in unserem Verständnis von visueller Wahrnehmung. In der vorliegenden Arbeit zeige ich, dass trotz grundlegender mechanistischer Unterschiede zwischen Seh- und und Hörvermögen beide Sinnessysteme Zugang zu Raumfrequenzinformation haben. Sechs Fledermäuse (*Phyllostomus discolor*) wurden darauf andressiert, eine Oberfläche mit Wellen unterschiedlicher Raumfrequenz und Tiefe von einer glatten Oberfläche zu unterscheiden. Meine Messungen zeigen dass Fledermäuse viel empfindlicher gegenüber hohen Raumfrequenzen sind als gegenüber niedrigen Raumfrequenzen, d.h. einen sensorischen Hochpassfilter für Raumfrequenz besitzen. Zusätzlich untersuchte ich welche sensorischen Reize den Fledermäusen zur Verfügung stehen um Raumfrequenz zu bewerten. Ich fand heraus, dass diese Reize sich grundlegend von solchen unterscheiden, welche die visuelle Wahrnehmung von Raumfrequenz vermitteln. Während visuelle Raumfrequenzwahrnehmung das Ergebnis feinabgestimmter räumlicher Empfindlichkeit der Retinazellen ist, wird Raumfrequenzwahrnehmung mit Echoortung durch objektspezifische Reflektionseigenschaften erreicht. Der Nachweis eines Hochpassfilters in der Echoortung von Fledermäusen offenbart funktionelle Gemeinsamkeiten zwischen Sehen und Echoortung, die beiden Systemen Zugang zum Raumprofil der Umgebung ermöglichen und damit der Figur-Grund-Wahrnehmung zugrunde liegen. Diese funktionellen Gemeinsamkeiten, aber mechanistischen Unterschiede machen deutlich, dass ein Sinnessystem-übergreifender Bedarf an räumlicher Umgebungsinformation besteht.

Das Gehör brilliert in der Messung winziger Laufzeitunterschiede. Doch wenn es darum geht zeitlichen Änderungen von Echoparametern zu folgen, scheint das Echoortungssystem einer typischen Fledermaus im Nachteil. Der Ortungsruf einer frequenzmodulierenden Fledermaus ist zu kurz um einen kompletten Bewegungszyklus abzubilden. Um Bewegung nachzuverfolgen müssen Fledermäuse die Laufzeitunterschiede ganzer Sequenzen von Ruf-Echo-Paaren vergleichen. Im zweiten und dritten Kapitel der vorliegenden Arbeit quantifizierte ich die Empfindlichkeit von Fledermausechoortung für zeitliche Modulationen verschiedener Echoparameter. Schlagende Insektenflügel erzeugen natürliche Echomodulationen, und zwar gleichzeitig Modulationen von Laufzeit und Lautstärke.

Im zweiten Kapitel führe ich eine Methode ein, mit deren Hilfe sich Laufzeit und Lautstärke der Echos unabhängig voneinander manipulieren lassen. Eine akustische virtuelle Realität ermöglicht die separate Untersuchung der Effekte des jeweiligen Parameters auf die Wahrnehmung der Fledermaus. Ich zeige, dass bei der frequenzmodulierenden Fledermaus *P. discolor* die Empfindlichkeit für Modulationen der Echolaufzeit stark von der Modulationsrate abhängt. Am empfindlichsten waren die Tiere bei Modulationsraten unter 20 Hz und über 50 Hz. Ich zeige, dass Echoortung für Wechselwirkungen zwischen Modulationsrate und Rufrate anfällig ist, ein Phänomen, das ich als einen echoakustischen Wagenradeffekt bezeichne. Weiter zeige ich, dass bei hohen Modulationsraten Doppler-Verzerrungen zusätzliche spektrale und zeitliche Reize herbeiführen, was den Wiederanstieg der Empfindlichkeit bei hohen Modulationsraten erklären kann. Die bedeutet, dass für die weltweit hunderten Arten frequenzmodulierender Fledermäuse Doppler-Verzerrungen eine wichtige Rolle bei der Flügelschlagwahrnehmung spielen könnten.

Im dritten Kapitel vertiefe ich meine Untersuchungen zum Thema Empfindlichkeit von Echoortung gegenüber Echomodulationen. Mit Hilfe der virtuellen Realität moduliere ich die Echolautstärke unabhängig von der Echolaufzeit. Ich kann zeigen, dass *P. discolor* diese Lautstärkemodulationen wahrnehmen kann und dass die Detektionsleistung der Tiere mit der Modulationsrate ansteigt. Ich führe an, dass sich die Wahrnehmung von Lautstärkemodulationen mit Echoortung grundlegend von der Wahrnehmung von Laufzeitmodulationen unterscheidet. Weiter spekuliere ich, dass der Wahrnehmung schneller Lautstärkemodulationen spektrale Reize zu Grunde liegen.

In ihrer Gesamtheit liefert die vorliegende Arbeit experimentelle Nachweise zu wichtigen perzeptorischen Prozessen in der Echoortung frequenzmodulierender Fledermäuse. Meine Erkenntnisse zeigen eine Möglichkeit auf, wie Fledermäuse dem vermeintlich unumgänglichen Kompromiss zwischen räumlichem und zeitlichem Auflösungsvermögen entgehen könnten. Damit stelle ich eine Alternative zur traditionellen Sichtweise, dass die sensorischen Einschränkungen des Gehörs automatisch zu geringerer Leistungsfähigkeit führen. Ich lege dar wie divers die Selektionsfaktoren sind, die auf das Echoortungssystem von Fledermäusen einwirken. Diese Dissertation nimmt daher Einfluss auf die Forschungsbereiche Neuroethologie, Verhaltensökologie, Tierphysiologie und Evolution, und kann zur Weiterentwicklung technischen Sonars beitragen.

SUMMARY

Bats emit ultrasonic cries and listen to the reflected sounds to orient and forage in their environment. The rich ecological niche of nocturnal air space became accessible through bats' capability of sustained flight and echolocation. Their "sixth sense" gained them autonomy from sunlight, but to what extent can hearing replace vision? This thesis addresses the question how echolocation encodes certain spatial and temporal parameters of the environment.

Echolocation poses a challenge to the perception of spatial layouts because the auditory sensory epithelium, the cochlea, does not explicitly encode space like the eye's retina does; space must be computed by comparing echo cues at both ears. In the first chapter of this thesis, I test the hypothesis that despite this challenge, bat echolocation utilizes the concept of spatial frequency to form perceptual representations of bats' habitat. Spatial frequency has been crucial to understand visual perception. I show that both sensory systems, echolocation and vision, have access to spatial frequency information despite their fundamental mechanistic differences. I trained six bats (*Phyllostomus discolor*) to discriminate ripples of different spatial frequencies from a smooth surface and measured echo-acoustic depth-contrast-sensitivity functions. I show that bats are much more sensitive to high spatial frequencies, exemplifying a spatial high-pass filter. Additionally, I evaluated the perceptual cues available to the bats to assess spatial frequency and found them fundamentally different from those in vision. While spatial frequency perception in vision is a result of spatial tuning, starting already in the retina, spatial frequency perception in echolocation is achieved by object-specific reflection properties that determine the perceived echo-acoustic object signature. The demonstration of a high-pass filter in bat echolocation reveals a functional similarity between vision and echolocation, which underlies figure-ground-separation and allows both systems access to the spatial contours in the environment. The functional similarities, yet mechanistic differences, highlight the need for spatial environmental information, independent of sensory system.

The auditory system excels in measuring minute differences in echo arrival times. But when it comes to the tracking of *changes* of echo properties over time, the echolocation system of a typical bat seems to be at a disadvantage. The echolocation call of frequency-modulating bats is too short to track an entire movement cycle. In order to track movement, bats have to compare memorised sequences of call-echo pairs. In the second and third chapters, I quantified the sensitivity of bat echolocation to the temporal modulation of echo parameters. In nature, fluttering insect wings cause echo modulations; the echoes carry modulations in echo delay and in echo amplitude simultaneously.

In the second chapter, I introduce an auditory virtual reality where I can manipulate delay independently from amplitude and tease apart the effects of both parameters on perception. I demonstrate that in the frequency-modulating bat *Phyllostomus discolor* the sensitivity for modulations in echo delay depends on the rate of the modulation, with bats being most sensitive at modulation rates below 20 Hz and above 50 Hz. I show that echolocation is susceptible to interference between call repetition rate and modulation rate. I propose that this phenomenon constitutes an echo-acoustic wagon-wheel effect. I further demonstrate how at high modulation rates sensitivity could be rescued by using spectral and temporal cues introduced by Doppler-distortions. Thus, I present evidence that Doppler distortions may play a crucial role in flutter sensitivity in the hundreds of frequency-modulating bat species worldwide. In the third chapter, I deepen my investigations into the sensitivity of bat echolocation to temporal echo modulations. I use the virtual reality approach to generate modulations in echo amplitude independent from echo delay. I show that *Phyllostomus discolor* successfully detected these modulations in echo amplitude and that their performance increased with the rate of the modulation. I suggest that amplitude-modulation detection with echolocation differs fundamentally from delay-modulation detection and speculate that the mechanism to detect fast amplitude modulations relies on spectral cues.

In summary, this thesis provides experimental evidence on important perceptual processes in the echolocation of frequency-modulating bats. I give a proof-of-principle demonstration offering release from the supposed trade-off between temporal and spatial acuity and challenging the view that the auditory system's sensory constraints inevitably lead to detrimental echo-acoustic performance. Thereby, my findings highlight the diversity of selective pressures working on the echolocation system of bats. This thesis therefore has implications on the fields of neuroethology, behavioural ecology, animal physiology and evolution, and may contribute to the further development of technical sonar.

INTRODUCTION

An early summer's evening in the foothills of the Alps. The sunset colouring the sky in purple and gold. A mild wind blowing up over the hills and across a small lake and its pebbly beach, creating soft waves on the water's surface. A short distance above, a swarm of small shadows shooting swiftly through the warm air. Every now and then, a single shadow swoops down to the water surface. No sound is to be heard over the backdrop of chirping crickets from the shore. Not to human ears, anyway. If we were to switch on a bat detector, however, a veritable cacophony of sounds would become audible.

Bats emit ultrasonic cries and listen to the reflected sounds to orient and forage in their environment. They echolocate. The unique combination of echolocation and sustained flight has opened the rich ecological niche of nocturnal air space to them. Where diurnal mammals use vision as their main remote sense, echolocation has evolved as the main remote sense in mammals that live under conditions with no external light. But to what extent can hearing replace vision? Or in other words, can sound really paint a picture?

The physical and anatomical principles underlying the two sensory systems are as different as they come. The brain, however, ultimately receives the same neural impulses in either case. This chapter aims to give an introduction first to the relationship between vision and hearing, then to the operating principles of echolocation and their physiological basis, next to the environmental parameters under study, and lastly to the scientific approach used throughout this thesis.

Vision vs. hearing - In order to understand the differences and similarities between vision and hearing, we will first look at their physical and physiological basis.

Light is electromagnetic radiation within a small portion of the electromagnetic spectrum. Vision refers to the ability to perceive the light in the visible spectrum reflected by objects in the environment and to interpret these reflections as images. Light is perceived via a chemical process in the receptor cells of the sensory epithelium of the eye, the retina. The explicit spatial layout of the retina and the fine spacing of photoreceptors support high spatial acuity and resolution along the two dimensions of azimuth and elevation. The perception of depth arises from computations in the visual cortex using binocular disparities, i.e., differences between the images of left and right eye. Depth-perception is prone to optical illusions.

Sound is a vibration that propagates as longitudinal wave of pressure in an elastic medium, e.g. air. Hearing refers to the ability to receive waves of certain wavelengths and interpret them as sounds. Sounds coming from any direction are superimposed at the eardrum and transmitted through the middle ear to the hearing organ of the inner ear, the cochlea. The cochlea contains the ear's sensory epithelium, the basilar membrane, where the (transformed) pressure wave mechanically stimulates the receptor cells. The basilar membrane is organized tonotopically, i.e., a certain place of stimulation codes for a certain sound frequency (pitch). This organization leaves the auditory system poorly set up for displaying space: spatial information must be neurally computed, for azimuth through binaural comparisons (Rayleigh 1907), and for elevation through the analysis of complex spectral interference patterns generated by the outer ears (Blauert 1997) .

As opposed to light, that usually originates from the sun and is basically inexhaustible while it travels through air, sound waves need a relatively nearby sound source because they become fainter over distance (geometric and atmospheric attenuation). In the case of echolocation, the bat itself is the sound source, emitting sounds of extremely high frequencies at ear-piercing volumes. The sound waves bounce off objects in the environments and return to the bat's ears with decreased volume, depending on the distance the sound has travelled but also on the reflection properties of the particular object. Hence the loudness of the echo, the echo amplitude, is a rather unreliable measure for object distance. A very dependable measure, however, is the time delay between outgoing call and returning echo, the so-called echo delay. This simple example already illustrates the operation principles of echolocation; the following section will trace the path of scientific findings that led to our present-day understanding of them.

The discovery of echolocation - The story of echolocation research begins in the 18th century. Italian physiologist Lazaro Spallanzani found that bats could avoid obstacles in flight not only in a dark room but even after they had been blinded. Spallanzani and his Swiss friend and colleague Louis Jurine eventually concluded that hearing was the sensory modality responsible for this feat, but they did not yet realise that self-emitted sounds played a crucial role. More than a hundred years later, American zoologist Walter L. Hahn (1908) repeated and extended their experiments and came to the same conclusion. Around the same time, American-British inventor Hiram Maxim (1912) proposed that bats used sound below the human auditory range to avoid obstacles. A few years later, English physiologist Hamilton Hartridge (1920) correctly proposed instead that bats used frequencies above the range of human hearing because only very short wavelengths would be reflected off the very small obstacles that bats were able to avoid in the dark. In occupied Holland of the 1930s, Dutch scientist Sven Dijkgraaf (1946) heard faint sounds from passing bats ("Ticklaute") and concluded that bats emitted sounds and used the echoes for orientation.

Also in the 1930s, Donald Griffin began studying the navigational methods of bats as an undergraduate at Harvard University. He knew about Spallanzani's and Hahn's findings, but not Hartridge's proposition of "supersound". Still, when he learned about the development of a microphone that could record ultrasonic frequencies, he pointed it at a cage full of little brown bats (*Myotis lucifugus*). Thus, Griffin and George W. Pierce, inventor of said microphone, made the first ever recordings of bat echolocation calls (Pierce and Griffin 1938), although at the time they still didn't know that this is what they were. They could barely record any sounds from flying bats. Only during his PhD work, Griffin and his fellow graduate student Robert Galambos pointed the Pierce microphone directly at flying bats and finally recorded the expected stream of ultrasonic pulses. They subsequently repeated and extended former experiments: they sealed bats' snouts shut and found them completely disoriented even when hearing was still intact. Griffin and Galambos thus proved that both the emission and the hearing of ultrasonic pulses are necessary for bats' sixth sense (Griffin and Galambos 1941; Galambos and Griffin 1942). Griffin (1944) eventually coined the term "echolocation". The expression proved to be not comprehensive enough, because bats can do more than just locate obstacles by means of echoes. Still, the term prevails, if rather for historical reasons than those of accuracy.

Operation principles of echolocation - Echolocation is always a trinity of call, target and echo (cf. Figure I.1A). Call properties and target properties together determine the properties of the echo. By comparing the call properties with the echo properties the bat can draw conclusions about the target properties.

The properties of a target are fully described by the target's acoustic impulse response (IR). The IR is the echo-acoustic counterpart of a visual image. As the visual image is a reflection pattern in response to a flash of white light, the IR describes the reflections of a 3D scene when it is ensonified with a spectrally white acoustic impulse of infinitely short duration and infinite amplitude (Dirac impulse, cf. Figure I.1B). Bats don't ensonify objects with theoretical acoustical impulses, but with echolocation calls produced with their vocal chords. Calls have a finite frequency spectrum, a finite duration, and finite amplitude. Convolving the actual call with the IR will recreate the echo as the bat would receive it. Vice versa, bats likely extract the IR from the echo in order to analyse the target properties (Weissenbacher and Wiegrebe 2003).

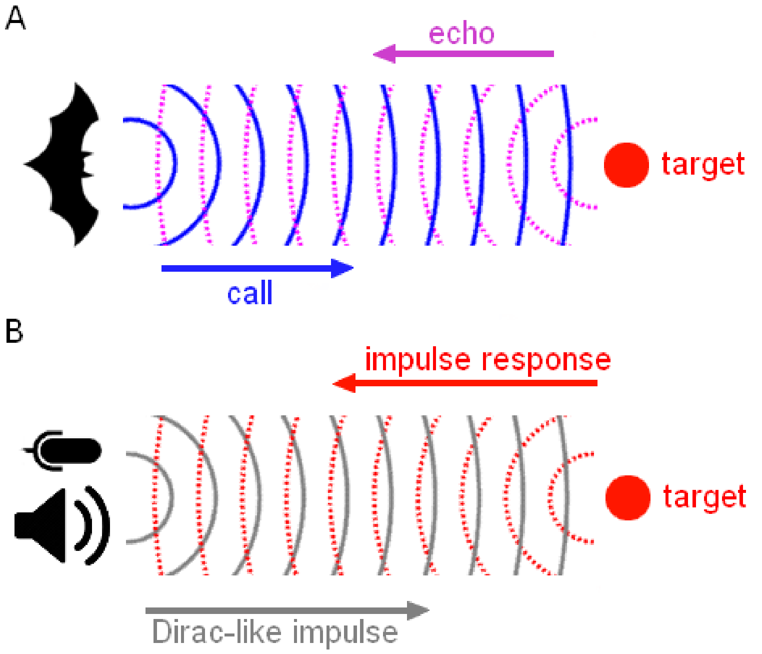

Figure I.1 Schematic of the operation principles of echolocation. *(A) A bat emits an echolocation call which is reflected off a target and returns to the bat as echo of the target. (B) When a target is ensonified with an acoustic impulse of infinitely short duration and infinite amplitude, the resulting reflection is the target's impulse response (IR).*

Static IRs describe static systems. Dynamic systems can only be described by dynamic, or time-variant, IRs. What makes a system dynamic? The answer to this question is, any change over time. The most common change over time is of course movement. A moving target is described by a time-variant IR.

The movement of a target can change the target's distance to the bat, or its reflection properties. For example, the fluttering wings of insects move back and forth, thereby changing the distance of the reflecting wing area. At the same time, the wings rotate, i.e. the size of the reflecting wing area changes, thereby changing the reflective strength (Griffin 1958; Roeder 1963). These changes in target properties are mirrored in changes within the time-variant IR: the echo-delay is modulated over time, and so is the target strength. Target strength is a measure of the target's reflective strength and is directly proportional to echo-amplitude. It is described by the quadratic mean of the target's IR. The modulations within the time-variant IR are carried over to modulations in the echo. How well they can be monitored within a single echo or a sequence of echoes depends on the design of the echolocation calls. In bats, two general types of echolocation calls have evolved: frequency-modulated calls (FM calls) sweep through a broad band of frequencies within a few milliseconds whereas constant-frequency calls (CF calls) keep a constant frequency over a much longer duration. Before we have a closer look at these two general types of echolocation we need to consider the physiological specialisations of sound perception and sound production in bats.

Hearing in bats – Hearing is an essential sense in all mammals. The functional anatomy of the bat's peripheral and central auditory system enables them to perceive frequencies above the human hearing range. However, the ability to hear ultrasound is not synonymous to the ability to echolocate. Many small mammals, e.g. mice, but also non-echolocating bats, show similar audiograms to echolocating bats (Neuweiler 1990). Bats show some physiological adaptations while following the general mammalian blueprint. The often-huge pinnae act as directional microphones. Additionally, they have an exceptionally well-developed tragus, the stiff skin fold at the opening of the ear canal. The tragus is essential for the determination of the elevation of a sound source relative to the bat (Lawrence and Simmons 1982; Wotton and Simmons 2000; Chiu and Moss 2007) and for controlling flight height (Hoffmann et al. 2014). Sound waves being reflected between tragus and pinna wall create interferences, i.e., the sound's frequency spectrum changes with relative vertical position. In CF species that use narrowband calls without much potential for spectral interferences, the tragus is poorly developed or missing (Neuweiler 1993). The bat's middle ear functions as a species-specific frequency filter: the tympanum is small and the ear bones are stiff and lightweight, amplifying high frequencies (Neuweiler 1993). Furthermore, the highly developed muscles of the middle ear contract whenever the bat emits a call and thus selectively suppress the perception of the bat's own emission (Jen and Suga 1976). The inner ear contains the cochlea where, as described above, the frequency decomposition is carried out. The cochlea is a coiled-up cavity inside the temporal bone of the skull, consisting of three fluid-filled canals. The third canal, the cochlear duct, hosts the Organ of Corti with the basilar membrane that carries the receptor cells. High frequencies maximally stimulate inner hair cells at the base of the basilar membrane; low frequencies maximally stimulate inner hair cells at its apex. Due to the mechanical properties of the basilar membrane the frequency decomposing happens on a logarithmic scale. As a rule of thumb, every octave covers an equally large portion of the basilar membrane. In a typical FM bat, the auditory filters thus widen with increasing sound frequency. In contrast, CF bats possess an auditory fovea: a very large portion of the basilar membrane is dedicated to the CF frequency, resulting in auditory filters of an extremely narrow bandwidth around that frequency (Kössl and Vater 1995). The tonotopy of the cochlea, i.e., its frequency-place relationship, is preserved along the entire auditory pathway up to the auditory cortex. Like all mammals, bats use spectral interference patterns that arise from the directional filtering of the outer ear for localizing targets along the vertical axis. These cues are monaural, as opposed to binaural cues that require comparing the inputs at both ears. For localizing targets along the horizontal axis, differences in sound arrival time or sound level between left and right ear are used: interaural time differences (ITDs) for low frequency sounds and interaural level differences (ILDs) for high frequency sounds (Moore 2012).

Vocal production in bats – Except for one genus of flying foxes (*Rousettus* spec.), whose echolocation signals consist of sharp tongue-clicks, all bats produce their echolocation calls following the general mammalian pattern, i.e., with their vocal chords in the larynx. But thanks to several adaptations of their vocal tract (i.e., laryngeal cavity, pharynx, oral and nasal cavity) they can emit ultrasonic frequencies at high intensities and high repetition rates. Their exceptionally thin vocal chords can vibrate at very high rates, generating very high frequencies (Novick and Griffin 1961): bats produce the highest tonal vocalizations known to date in the animal kingdom, with frequencies reaching 250 kHz (Schmieder et al. 2010). Many tonal sounds consist of several harmonics, i.e., sound components with a frequency that is an integer multiple of the frequency of the fundamental frequency. The members of some bat families (Phyllostomidae, Rhinolophidae, and Hipposideridae) increase the effective frequency of their emissions by suppressing energy in the fundamental and in lower harmonics with their vocal tract (Au and Suthers 2014). Similarly, they can increase their call bandwidth by distributing energy to more than one harmonic, resulting in multiharmonic echolocation calls. Bats emit their calls through their mouth, their nostrils or both (Seibert et al. 2015). This focuses the main energy of the call forwards into a sound beam. Within species-specific limits, bats can broaden or narrow the shape of their sonar beam according to the task at hand (Linnenschmidt and Wiegrebe 2016; Jakobsen et al. 2018).

FM and CF echolocation and the duty-cycle - Echolocation is an active sense, i.e. bats need to actively emit sound in order to receive echoes instead of passively listening to sounds produced by others. Aside from motor-performance constraints (Elemans et al. 2011), the portion of time that a bat can call is limited by the fact that it also needs to listen for the returning echo after each emission. The longer the call, the longer the bat is forced to pause in order to avoid overlap of call and echo (but see CF echolocation below). Thus, the duty-cycle of echolocation is never 100 %. There are two distinct groups of bats, one that emits calls at less than 25 % of the time (low duty-cycle) and one that emits calls at more than 25% of the time (high duty-cycle) (Neuweiler and Fenton 1988; Fenton 1995). This grouping is in accord with the two echolocation call designs mentioned above, FM and CF echolocation calls.

Frequency-modulating bats (FM bats) constitute more than 80 % of all echolocating species. Their calls sweep through a wide band of frequencies within few milliseconds. The exact structure of the call varies with the task that the bat is facing. A typical foraging sequence can be divided into search, approach and capture. The emissions in every phase vary in duration, repetition rate, and frequency content. Search calls are often longer than 10 ms and relatively narrowband with a shallow frequency modulation. But call design also depends on the habitat the bat is foraging in. Amidst dense vegetation calls will be fainter, shorter and more steeply modulated than in the open air in order to avoid distraction through non-prey echoes, so-called clutter echoes. After a bat has detected a potential prey item, it will emit approach calls of shorter duration and wider bandwidth, resulting in a steeper frequency modulation, at higher repetition rates. The task has now changed from detection to localisation and identification of prey. The final stage is characterised by a very rapid series of very short calls that consecutively decrease in frequency. This sequence of calls is referred to as final or terminal buzz. The emission of a buzz is reflex-like and sometimes only ends after the actual capture of the prey (Geberl et al. 2015). Some FM species emit a final buzz also when landing on a surface, while others do not use it at all (Ratcliffe et al. 2013).

CF echolocation independently evolved in bats belonging to the families of Rhinolophidae and Hipposideridae as well as in one species of Mormoopidae (*Pteronotus parnellii*) (Teeling et al. 2005; Tsagkogeorga et al. 2015). CF signals consist of constant frequency tones. The species-specific CF frequency matches the bat's auditory fovea, so that smallest deviations in the echo's frequency can be detected. Fluttering wings or prey movement relative to the bat introduce tiny Doppler shifts, i.e., broadenings of the echo's spectrum, and simultaneous peaks in echo amplitude (Schnitzler and Denzinger 2011; Schoeppler et al. 2018; Suga 2018). These momentary effects are sometimes called acoustic glints, in analogy to light glints reflecting off moving objects. They become apparent within one single echo because CF calls are exceptionally long; call duration in search flight averages from 5-20 ms in Hipposiderids to 50–80 ms in Rhinolophids (Denzinger and Schnitzler 2013). Echoes reflected off nearby targets will thus overlap with the call, as echoes from vegetation will overlap with echoes from prey. The bat can ignore this, however, as long as the echo doesn't carry the typical glints that signal fluttering insect wings.

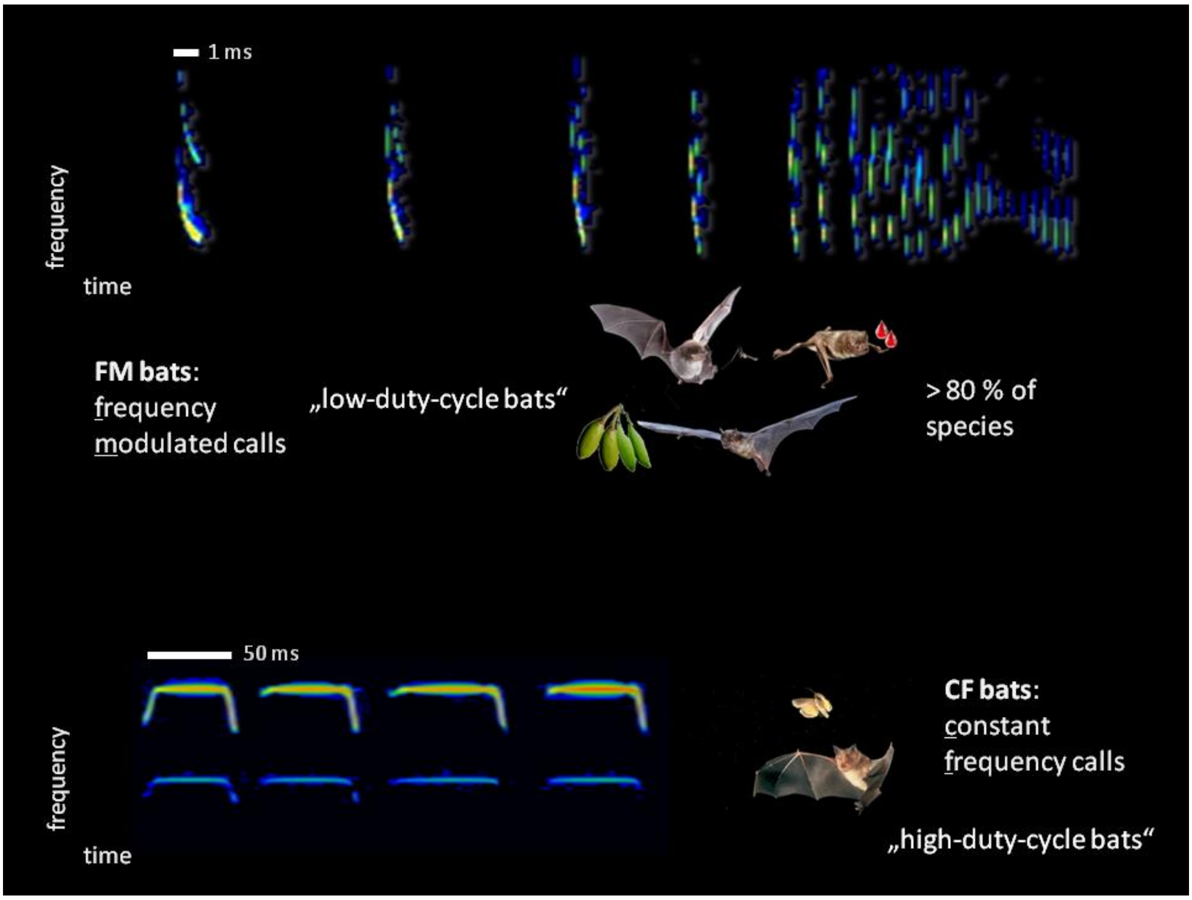

FIGURE I.2 COMPARISON OF FM AND CF BATS Frequency-modulating bats constitute more than 80% of all bat species, covering different foraging ecologies. Their echolocation calls are very short and broadband. FM bats are also called low-duty-cycle bats because of the short duration of the calls in relation to the inter-call intervals. From left to right, the calls in the depicted sequence illustrate the three phases search, approach and buzz. In contrast to FM bats, constant-frequency bats are often specialized to hunt fluttering insects with their rather long echolocation calls that contain one prominent frequency-band, but often also frequency-modulated parts (upwards or downwards modulated). The silent intervals between calls are much shorter, therefore CF bats are also known as high-duty-cycle bats. Bat photos by D. Nill

The spatial resolution at which a target is depicted increases with the range of frequencies an echolocation call covers, the call bandwidth. The temporal resolution, at which changes in a target are depicted, however, is limited by the duration of the call. In comparison to a CF call, a broadband short FM call grants high spatial resolution at the expense of accuracy in tracking the movement of the target. However, we have to keep in mind that here spatial resolution refers to depth-resolution, not to resolution along the azimuth/elevation axes as we are used to in vision. The following sections will illuminate spatial and temporal parameters of the environment and how they are depicted with echolocation.

Spatial frequency – Since the pioneering work of Wiesel and Hubel on simple and complex receptive fields (Wiesel and Hubel 1966), the concept of spatial frequency has proven one of the most powerful tools to understand visual perception. Spatial frequency is a measure of change per unit space. A basic stimulus for visual research is the sine-wave grating, a repeating sequence of light and dark bars, where the light intensity changes sinusoidally (cf. Figure 1.1A). Gratings can vary in four independent parameters: contrast, orientation, phase, and spatial frequency. The contrast of a grating is defined as the maximum luminance minus the minimum luminance divided by twice the mean luminance ($\frac{L_{max}-L_{min}}{2\bar{L}}$; Michelson 1927). Spatial frequency refers to the number of light-and-dark cycles per meter (cyc/m). The contrast needed by any visual system to perceive a light-and-dark grating depends on spatial frequency and on the distance from which the grating is viewed: the contrast sensitivity function describes the just-detectable (perceptual or neural) contrast as a function of spatial frequency (Enroth-Cugell and Robson 1966; Campbell and Robson 1968) where the latter is converted to grating cycles per degree of visual angle (cyc/deg).

High spatial frequencies, i.e. steep changes of intensity along one spatial axis, represent local object features (e.g., object edges) whereas low spatial frequencies code for more global information about the object’s shape (Bar 2004). In images that contain both low and high spatial frequencies at similar proportions, the high frequencies are perceived as the figure and the low frequencies as background. The larger the spatial frequency difference, the more pronounced is the perceptual figure/ground separation (Klymenko and Weisstein 1986).

For echolocating animals, one potential equivalent of a light-and-dark grating is a surface that changes its depth periodically (cf. Figure 1.1A): In echolocation, depth is explicitly encoded through the measurement of the echo delay (Simmons 1971; Simmons 1973; Simmons et al. 1979) and, like azimuth and elevation in the visual system, it is topographically represented in the bat auditory cortex (O'Neill and Suga 1979; Hoffmann et al. 2008; Hagemann et al. 2010). Natural depth gratings occur for instance on water surfaces when the touch of an object, such as a raindrop, creates spreading ripples.

The spatial frequency of such surface waves depends upon mass and surface tension. Short waves have a high spatial frequency. The wave period is smaller than the sonar beam's diameter, the sonar footprint (Vanderelst et al. 2010). The different echo delays within the sonar footprint will create a complex interference pattern that is projected upon the bat's tonotopic frequency axis. The spatial assessment may be further refined through binaural interaction and the dynamic components of both the sending and receiving system (Mogdans et al. 1988; Ghose and Moss 2003; Holland and Waters 2005; Morinaka et al. 2007; Surlykke et al. 2009). Long surface waves have a low spatial frequency. The wave period may well be larger than the size of a bat's sonar footprint. In response to a single echolocation call, a bat will thus get the same echoes as from a smooth surface without waves. Long waves can only be perceived through the changes of echoes (possibly in terms of echo delay) across a sequence of calls. Therefore, long waves require the analysis of temporal frequency.

Temporal frequency – Temporal frequency is a measure of change per unit of time. How many periods of a periodic event fit into one time unit? The analysis of temporal frequencies has been intensively studied with regard to the analysis of echoes generated from fluttering targets like flying insects. We already know that in this case, the echoes are time-variant because of the movement of the ensonified target. The fluttering wings generate a modulation of both echo delay and echo amplitude over time (Neuweiler 1984; Schnitzler et al. 1985). Bats' ability to detect modulations of echo parameters is often referred to as flutter sensitivity. Sensitivity to flutter in CF bats has been investigated extensively (reviewed in Neuweiler 1990), both in theoretical studies (Kober and Schnitzler 1990) and in experimental studies (Schnitzler and Flieger 1983; Von der Emde and Schnitzler 1986; Von Der Emde and Menne 1989; Von der Emde and Schnitzler 1990; Roverud et al. 1991; Koselj et al. 2011). In CF bats, the call duration is much longer than the temporal- frequency period, allowing for a continuous monitoring of amplitude changes (Gustafson and Schnitzler 1979; Schnitzler and Flieger 1983; Von der Emde and Schnitzler 1986; Kober and Schnitzler 1990; Von der Emde and Schnitzler 1990; Tian and Schnitzler 1997).

In FM bats, the nature of echolocation is more stroboscopic: call durations do not exceed a few milliseconds and thus variations in the echo must be sampled across a sequence of echolocation calls. Flutter sensitivity in FM bats has been addressed by a mere handful of studies. The behavioural studies by Sum and Menne (1988), by Roverud et al. (1991) and by Grossetete and Moss (1998) investigated it in terms of discriminating one flutter-rate from another, but not in terms of how large a flutter needs to be at a given flutter rate so that it can be detected by the bat.

Sensitivity to the magnitude of delay changes was examined in the classical phantom-target jitter experiments by Simmons (1979) that were repeated by Menne et al. (1989). However, these experiments were not originally designed to assess flutter sensitivity and therefore the temporal frequency of the modulation was not studied as an independent parameter: the modulation was rectangular, i.e., jumped from one state to another with every emitted call. In echoes that result from fluttering insects, the modulation is sinusoidal and independent of the bat's ensonification. Only one study has investigated bats' sensitivity to the magnitude of echo delay modulation for a fixed, call-independent temporal frequency: Goerlitz et al. (2010) trained free-flying bats to discriminate between a stationary loudspeaker membrane and a membrane sinusoidally vibrating at 10 Hz. The perceived call-to-call flutter depended on call rate and call emission time in relation to modulation phase.

Before we turn to the aims of this thesis, I will briefly introduce the scientific approaches central to this study: the use of psychophysical methods and the implementation of an auditory virtual reality.

Psychophysics – Psychophysics is the study of perception with behavioural methods. German philosopher, psychologist and physicist Gustav Fechner founded the field in 1860. His famous Weber-Fechner-law describes the relationship between a physical stimulus and the according sensation. More specifically it describes the relation between the actual change in a physical stimulus and the perceived change. Perception of a stimulus often changes with some stimulus variable. For instance, the auditory threshold for detecting a faint sound varies depending on the sound's frequency (pitch). When we plot perception thresholds as the function of some stimulus dimension, we obtain a psychophysical function. In this example, we plot hearing thresholds against sound frequency and obtain an audiogram. To measure the thresholds in the first place, we collect psychometric data. A psychometric function describes the relationship between the stimulus magnitude and the subject's response. The just-noticeable difference (JND) in stimulus magnitude yields the threshold value. In the audiogram example, we play sounds of a certain frequency at varying volumes and record the subject's response: can you hear the tone or not? The just noticeable volume (i.e., the difference to silence) is the hearing threshold for that tone frequency. In order to make these tests more objective and prevent cheating, the subject must prove that they can detect the stimulus, for example by stating from which earphone the sound was being played. We refer to this paradigm as two-alternative, forced choice (2AFC). When working with animals, we can use 2AFC to assess their responses: Animals are offered a choice between a reference stimulus (e.g. silence) and the test stimulus (e.g. a loud tone) and trained to choose the reference stimulus for a food reward. They indicate their choice by approaching the one food source among two alternatives that is associated with the reference. Once they discriminate the reference from the test with a stable performance rate, training is completed and measuring begins. The difference between reference and test stimulus is decreased (the tone is played progressively fainter) until the animal is left to guessing which one is which: discrimination performance drops to chance level. The just-noticeable difference between reference and test stimulus yields the perception threshold (the hearing threshold for the measured tone frequency) (cf. Sekuler and Blake 1985).

Virtual reality - The stimuli presented in psychophysical experiments can be real objects or they can be virtual representations thereof. In the case of echolocation, we can make use of virtual echoes to simulate virtual targets. We create these virtual echoes by taking an emitted echolocation call and convolving it with an impulse response (IR) that carries the properties of the desired target. As we know, the IR is like a filter whose characteristics are determined by the physical properties of the target (cf. Figure I.1B). With modern processors that can operate in real-time at high sampling-rates, we can use time-variant IRs to create dynamic virtual targets. The important advantage of a time-variant IR is that it truly simulates a moving target: it produces an echo with the target properties at the specific moment in time when the call is emitted, so that it interacts with call properties like call duration and inter-call interval, affecting echo frequency, duration and delay. Like real moving targets, time-variant impulse responses can thus create e.g. Doppler distortions and echo-amplitude modulation.

AIMS OF THE THESIS

In our opening example, bats were hunting on a small lake during springtime. Many bat species from at least three families have specialized in trawling prey from water surfaces (Kalko et al. 1998; Ruedi and Mayer 2001; Weinbeer et al. 2006). Smooth water surfaces are considered clutter-free (Kalko and Schnitzler 1989) and may even facilitate prey detection due to the acoustic mirror effect: the echoes from the prey item are also mirrored by the water surface, thereby increasing the echo amplitude (Siemers et al. 2005). A turbulent water surface on the other hand reflects the sonar emissions back towards the bat, creating background echoes that may impair prey detection (Von Frenckell and Barclay 1987; Mackey and Barclay 1989; Rydell et al. 1999). Indeed, trawling bats prefer smooth water to turbulent or vegetation-covered water when foraging (Von Frenckell and Barclay 1987; Mackey and Barclay 1989; Boonman et al. 1998; Rydell et al. 1999). In contrast to these findings, a more recent study (Zsebők et al. 2013) indicates that prey detection of the European trawling bat *Myotis daubentonii* is not impaired by turbulent water. The discrepancy of these results suggests that the degree of turbulence determines the degree of prey detection impairment. Low degrees of turbulence result in low spatial frequencies, because the surface tension of water acts as a spatial low-pass filter, smoothing out sharp edges. High degrees of turbulence result in high spatial frequencies, as well as protruding solid structures (e.g. duckweed) and movement on or below the surface (prey).

All water surfaces are characterised by their co-varying spatial and temporal frequencies. The overall goal of this thesis is to quantify the sensitivity of bat echolocation to spatial and temporal frequency.

Chapter 1 tests the hypothesis that despite the fundamental anatomical and mechanistic differences between vision and hearing, bat echolocation analyses spatial frequency to perceive three-dimensional spatial layouts. We measure the echo-acoustic depth-contrast sensitivity functions of six pale spear-nosed bats (*Phyllostomus discolor*) in a classical psychophysical experiment. We further analyse impulse responses of artificial wave surfaces to gain insight into the perceptual cues available to the bats.

Chapter 2 tests the hypothesis that for evaluation of changes across entire sequences of call-echo pairs the relation between call rate and modulation rate plays a crucial role. Therefore, we quantify bats' sensitivity to the modulation of echo delay in a psychophysical experiment with six pale spear-nosed bats (*Phyllostomus discolor*). We use a virtual reality to manipulate the modulation of echo delay independently from the modulation of echo amplitude. We analyse bats’ emissions and measure echo properties to elucidate the underlying neural processing mechanisms.

Chapter 3 aims to answer the question whether the relation between call rate and modulation rate is independent of the nature of the modulated stimulus. Echo delay and echo amplitude are encoded differently in the bat ascending auditory pathway (Hagemann et al. 2010; Hechavarría et al. 2013; Greiter and Firzlaff 2017; Measor et al. 2018). We investigate whether this might also be true for the encoding of modulations of the co-occurring echo features. Again, we implement a virtual reality in a psychophysical experiment with four pale spear-nosed bats (*Phyllostomus discolor*). We modulate echo amplitude independently from echo delay. Further, we analyse bats’ emissions and simulate echo properties to establish possible perceptual cues.

CHAPTER 1

Bat echo-imaging perceives spatial frequency with a non-spatial sensor

Spatial frequency analysis is fundamental to visual tasks such as discrimination of objects from background. The retina's layout directly encodes space; the auditory system, in contrast, needs to compute all spatial information. Here we demonstrate that the auditory system nevertheless processes spatial frequency with a spatial high-pass filter directly comparable to vision. Six echolocating bats were trained to discriminate gratings of different spatial frequencies from a flat surface in darkness. Bats were much more sensitive to high rather than low spatial frequencies, likely mediated by the gratings' reflection properties. These functional similarities, yet mechanistic differences between vision and hearing illustrate convergent evolution of spatial information processing and challenge the notion that the auditory system's sensory constraints inevitably lead to inferior spatial performance.

A. Leonie Baier, Lutz Wiegrebe, and Holger R. Goerlitz Bat echo-imaging perceives spatial frequency with a non-spatial sensor.

Bat echo-imaging perceives spatial frequency with a non-spatial sensor

The main remote sense in diurnal mammals is vision, whereas nocturnal mammals (and those living in murky waters) evolved sound-based echo-imaging. Both systems' main task is to delineate the layout of far space in 3D for orientation and navigation. While the retina's two-dimensional anatomy supports high spatial acuity and resolution, the auditory system is poorly set up for displaying space: incoming sounds are not mapped onto a two-dimensional projection of the world, but superimposed at the eardrums and then decomposed along a frequency-axis by the inner ear. Spatial information must be neurally computed (Rayleigh 1907). For echo-imaging, bats use the returning echoes of their ultrasonic calls to analyze objects within the emitted sound beam. They compute an object's structure along the azimuth, elevation and distance axes in the central auditory system from the spectro-temporal echo cues at both ears (Simmons 1971; Lawrence and Simmons 1982; Simmons et al. 1983; Schmidt 1988b; Firzlaff et al. 2007).

Spatial frequency quantifies change as a function of position, e.g. changes in brightness along azimuth. Spatial frequency analysis is fundamental to visually perceiving the environment (Wiesel and Hubel 1966). High spatial frequencies characterize local object features (object edges), and low spatial frequencies characterize global object features (object shading) (Bar 2004). For figure-ground separation, humans perceive high frequencies as the figure and low frequencies as background; the larger the difference in frequency, the clearer the perceived separation (Klymenko and Weisstein 1986). A basic stimulus for investigating visual spatial frequency analysis is the sine-wave grating, a repeating sequence of light and dark stripes where the light intensity changes sinusoidally along one spatial axis. For echolocating animals, one potential equivalent of a light-and-dark grating is a surface that periodically changes its depth (Fig. 1.1A). Depth is explicitly encoded through echo delay (e.g. (Simmons 1973)) and is topographically represented in the bat auditory cortex (e.g. Hoffmann et al. 2008) like azimuth and elevation in the visual system. Natural depth gratings occur for instance on water surfaces when the touch of an object (raindrop, insect) creates spreading ripples (cf. Fig. 1.1B).

Here we hypothesize that despite the fundamental anatomical and mechanistic differences between vision and hearing, bat echo-imaging analyses spatial frequency to perceive 3D spatial layouts. Spatial frequency sensitivity in vision is quantified by the contrast sensitivity function, which describes the (perceptually or neurally) just-detectable contrast as a function of spatial frequency, i.e., the contrast needed by any visual system to perceive a light-and-dark grating (Campbell and Robson 1968). In accordance to the visual contrast sensitivity function, we measured the echo-acoustic depth-contrast sensitivity functions of six pale spear-nosed bats (*Phyllostomus discolor*) in a classical psychophysical experiment.

Bats were trained in a two-alternative, forced-choice paradigm with food reward to choose between a flat reference disc and a disc with concentric grating of varying depth and spatial frequency (Fig. 1.1C). After bats discriminated a large grating depth of ±16 mm at a spatial frequency of 0.5 cyc/deg to criterion (70% correct), we stepwise reduced grating depth, collecting 30 trials per grating depth. The experiments yielded one psychometric function per bat, i.e., the discrimination performance as a function of grating depth (Fig. 1.2A). We then measured psychometric functions for four more spatial frequencies. Due to production limitations, we could not investigate spatial frequencies higher than 2 cyc/deg. For all bats and across all spatial frequencies, discrimination performance was poor at low grating depths and improved with higher grating depths (Fig. 1.2A), For instance, at a spatial frequency of 0.5 cyc/deg, grating depths up to ±1 mm were not discriminated from a flat disc above chance level. Discrimination improved as grating depth increased, reached threshold level at ±4 mm in five out of six bats, and improved further with the larger grating depths of ±8 and ±16 mm. Thus, echo-imaging is more sensitive to deeper gratings than to shallow gratings. This pattern existed for all spatial frequencies, yet with frequency-dependent differences: while at the lowest presented spatial frequency (0.125 cyc/deg) none of the bats detected even the strongest depth modulation (±16 mm), the bats reliably detected ±1 mm gratings at the highest spatial frequency of 2 cyc/deg. We estimated actual discrimination thresholds for each spatial frequency and animal from a fitted sigmoidal function at

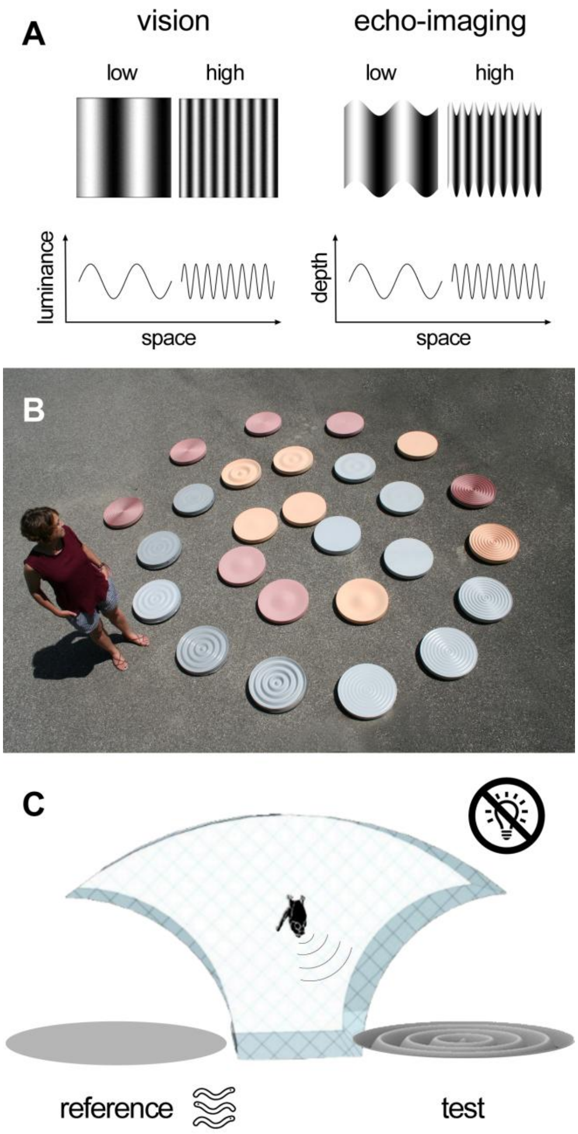

FIGURE 1.1 SPATIAL FREQUENCY IN VISION AND ECHO-IMAGING. *(**A**) Spatial frequency quantifies the periodic change of either luminance (vision) or depth (echo-imaging) along a spatial axis, expressed in units of cycles per degree observation angle. (**B**) We simulated natural depth gratings with discs covered in concentric depth gratings. (**C**) Six bats discriminated in darkness between the flat reference disc and the test disc with depth gratings of varying depth and spatial frequency. Bats indicated the pseudo-randomly chosen position of the flat disc by crawling towards it from the starting position after echolocating towards both discs.*

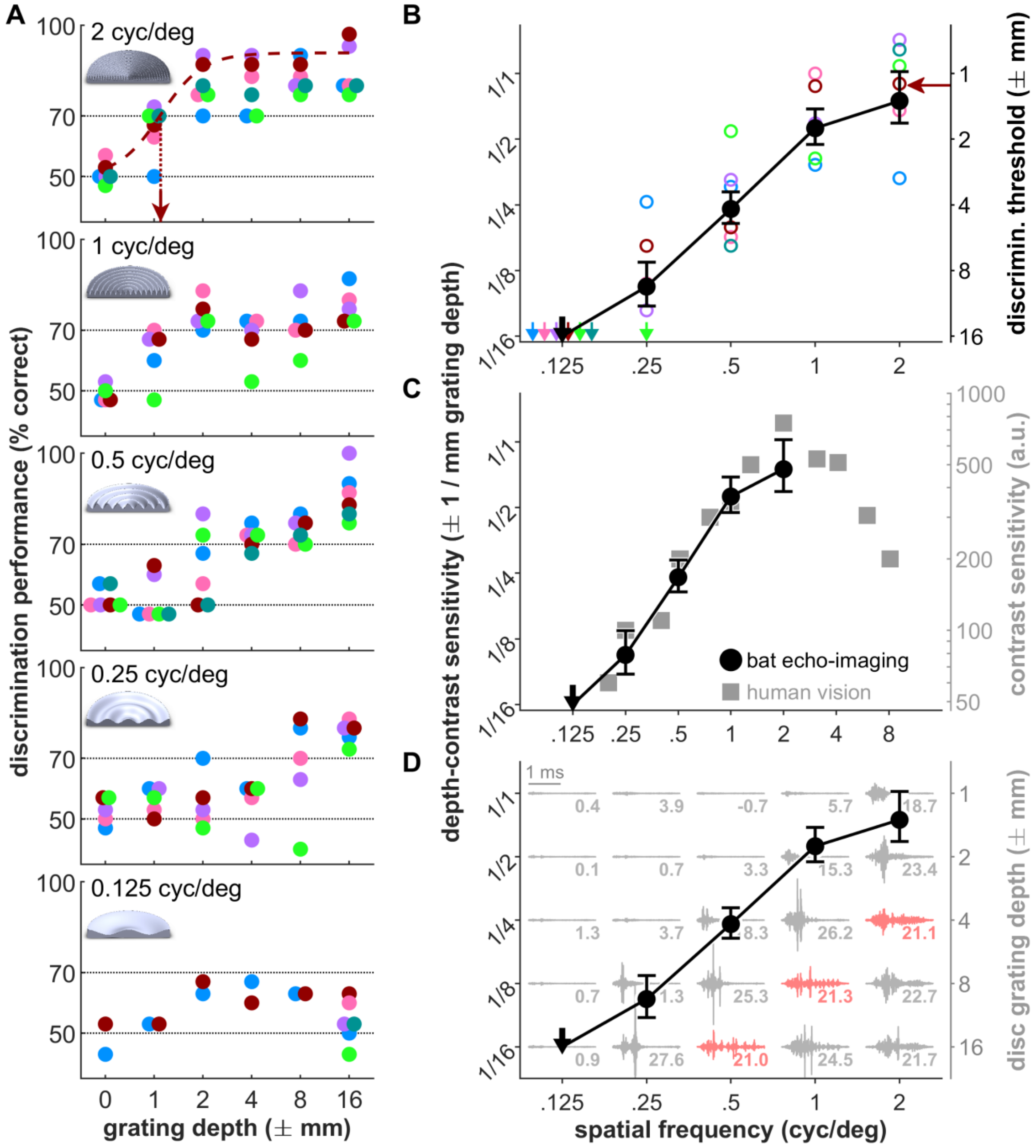

*FIGURE 1.2. DEPTH-GRATING DISCRIMINATION REVEALS A SPATIAL HIGH-PASS FILTER IN ECHO-IMAGING. (**A**) Depth-grating discrimination performance of six bats (colored dots, n=30 trials per dot) as a function of grating depth at five spatial frequencies. Discrimination thresholds were extracted from the fitted psychometric functions at 70% correct (exemplary sigmoid dashed line and arrows in A and B). Horizontal dotted lines at 50% and 70% correct depict chance and significance ($p<0.05$) levels, respectively. Insets show exemplary grated discs with ±16 mm grating depth. (**B**) Individual (colored) and mean (black, ± s.e.m.) depth-contrast sensitivity (the reciprocal of depth-contrast threshold, as extracted from A) improves with increasing spatial frequency, forming a spatial high-pass filter. Where no threshold was found within the range of tested grating depths (downward-pointing arrows), threshold was assumed to be higher than ±16 mm, but set to ±16 mm for calculations. (**C**) The high-pass characteristic of the current depth-contrast sensitivity in bat echo-imaging (black circles) is well comparable to the luminance-contrast sensitivity in human vision (grey squares: black-and-white contrast sensitivity, adapted with permission from Campbell & Robson 1968[11]). (**D**) The mean depth-contrast sensitivity function overlaid on the measured acoustic impulse responses (aIR) of all grated discs. Amplitude and duration of aIRs increase with increasing grating depth (top to bottom) and spatial frequency (left to right). Small numbers indicate target-strength difference (TSD) between each grated disc and the reference disc. Depicted in red are exemplary aIRs of different spatial frequency and grating depth but not target strength; these aIRs differ in their temporal structure.*

70% correct performance ($p<0.05$, binomial test; cf. Fig 1.2A, dashed red line). Combined, the extracted threshold values form the depth-contrast sensitivity function, with depth-contrast sensitivity being the reciprocal of the discrimination threshold (Fig. 1.2B). Depth-contrast sensitivity improved with increasing spatial frequency: all bats required deeper gratings to detect low spatial frequencies, while shallower gratings sufficed with increasing spatial frequencies. This high-pass characteristic is qualitatively comparable to the high-pass portion of the human visual contrast sensitivity function (Fig. 1.2C).

To understand the auditory perceptual cues behind the bats' ability to assess spatial frequency, we measured the acoustic impulse responses (aIR) of the depth gratings from the bats' perspective. The aIR is the echo-acoustic counterpart of a visual image. As the visual image of an object is a reflection pattern in response to a flash of white light, the aIR is the reflection pattern in response to a spectrally white acoustic impulse. It fully describes the object's reflection properties and determines the returning echo. Amplitude and duration of the gratings' aIR (and therefore also their echoes) increased with increasing grating depth and spatial frequency (Fig. 1.2D). We estimated relative echo amplitude by calculating the difference in target strength (root mean square of the aIR) between each grated test disc and the flat reference disc. These biophysical measurements parallel the perceptual thresholds: target-strength differences (TSD) increased with increasing grating depth and spatial frequency, with a very sudden increase around the grating-depth threshold (Fig. 1.2D). The discs' TSD at discrimination threshold for each spatial frequency roughly matches *P. discolor*'s threshold for TSD of about 5-7 dB (Heinrich et al. 2011), suggesting that spatial frequency sensitivity is mediated by TSD (Fig. S1). However, to discriminate spatial frequencies with equal target strengths, bats will require additional cues. These cues might be distinct temporal envelope structures (compare colored aIRs in Fig. 1.2D), which are well perceptible to bats (Grunwald et al. 2004).

To date, we understand very little about the perception of complex 3D layouts with echo-imaging (Wohlgemuth et al. 2016). Our results provide an important demonstration of the auditory systems' capabilities for spatial perception as basis for orientation and navigation.

First, we demonstrated a perceptual spatial high-pass filter in echo-imaging that allows extracting the contours of objects, directly comparable to vision. This convergent evolution across sensory systems points to a strong survival benefit of perceiving the spatial layout of the environment. Spatial-frequency mediated figure-ground-separation would facilitate prey detection in complex auditory scenes by rejecting low-frequency background and letting pass high-frequency prey information. One relevant scenario is the detection of insects on or above water surfaces ("trawling"). Despite the background echoes produced by surface turbulences, trawling is common in bats (Kalko and Schnitzler 1989; Schnitzler et al. 1994; Weinbeer et al. 2006). It is possibly promoted by spatial high-pass filtering: weak turbulences, like waves, possess low spatial frequencies, because the surface tension smoothes out sharp edges. Protruding solid structures (prey) and strong turbulences generated by prey movement possess high spatial frequencies. Similarly, a perceptual spatial high-pass filter will promote the detection of prey on smooth vegetation surfaces (Geipel et al. 2013) as well as of roosts (Ruczynski et al. 2007), since the sharp edges of holes, cracks and other openings in trees and rock surfaces increase the spatial frequency content.

Second, we demonstrated that high-pass filtering in echo-imaging is likely mediated by conversion of spatial features into proximate echo parameters (amplitude, duration, envelope fluctuation), which then undergo established auditory analysis. In the primate visual system, contrast sensitivity is mediated by the spatial arrangement of neuronal connections in the retina, mechanistically implemented by the center-surround organization of the receptive fields of ganglion cells (Hartline 1949; Von Bekesy 2017). The size of its receptive field determines the cell's response to gratings of different spatial frequencies and the cut-off-frequency of its spatial high-pass filter: spatial frequencies whose period exceeds the size of the receptive field are hard to perceive. Consequently, the effective functional similarity of spatial high-pass filtering in vision and echo-imaging, allowing figure-ground separation in both systems, must not be extrapolated to the spatial resolution of both sensory systems. Due to the mechanistic differences, echo-imaging will not provide a resolved perceptual representation of gratings within the pass-band of the spatial high-pass filter.

Our findings suggest a mechanistic sensory strategy for converting spatial layout into perceptible auditory cues. They highlight how selective pressures have convergently shaped both vision and audition to extract spatial features for orientation and navigation, and challenge the notion that the auditory system's sensory constraints inevitably lead to inferior spatial performance.

SUPPLEMENTARY MATERIALS

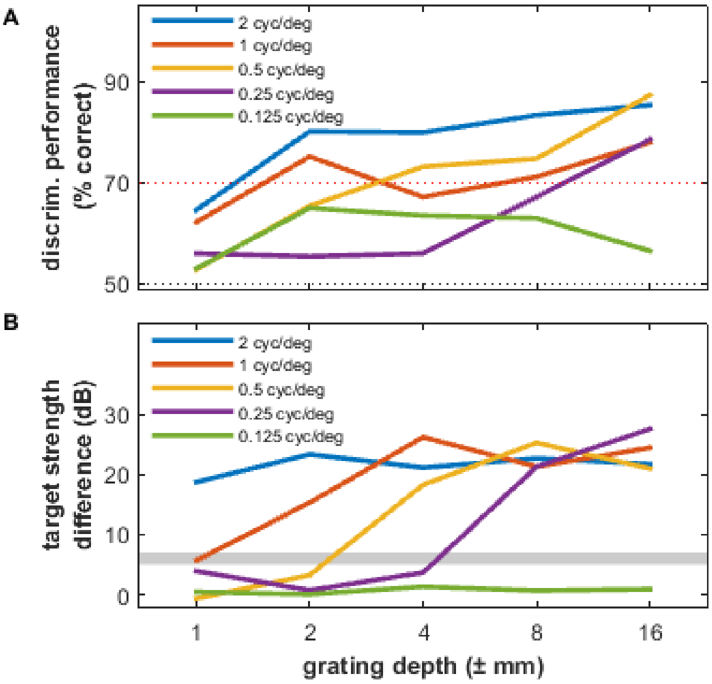

FIGURE S1 DIFFERENCES IN TARGET STRENGTH CAN SERVE TO EXPLAIN THE BATS' DETECTION PERFORMANCE. *(**A**) Each line depicts detection performance (mean over all bats) at the denoted spatial frequency as a function of grating depth. Horizontal dashed lines at 50 and 70 % correct depict chance and significance level, respectively. (**B**) Each line depicts the target-strength difference (at 45°ensonification angle) between the test disc of the denoted spatial frequency and the reference disc as a function of the test disc's grating depth. Grey bar depicts target-strength-difference-discrimination threshold (Heinrich et al. 2011).*

Experimental animals We used six adult individuals (four male, two female) of the neotropical omnivorous bat species Phyllostomus discolor, Wagner, 1843.These bats emit short (<3 ms), downward frequency-modulated, multi-harmonic echolocation calls covering the frequency range between 45 and 100 kHz (Rother and Schmidt 1982). Bats originated from a breeding colony at the Department Biology II of the Ludwig Maximilians University Munich and were kept at the Max Planck Institute for Ornithology in Seewiesen (12 h night / 12 h day cycle, 65-75% relative humidity, 28°C) with unlimited access to water at all times. On free days, the bats had ad libitum access to mixed fruit and mealworms (larval form of Tenebrio molitor) supplemented with essential fatty acids (Efaderm®), minerals (Korvimin ZVT+Reptil®) and vitamins (NutriCal®). During training periods, bats were fed with mealworms in the experiment. All experiments complied with the principles of laboratory animal care and were conducted under the regulations of the current version of the German Law on Animal Protection (approval 55.2-1-54-2532-34-2015, Regierung von Oberbayern).

Stimuli The 27 stimulus discs (Fig. 1.1B) were manufactured by a milling cutter (Modellbau Grossmann, Calw). They were 5 cm high, 45 cm in diameter wide and were covered in concentric waves. From the bat's typical viewpoint in the setup (40 cm decision distance and 45° ensonification angle), the discs covered a considerable area of the bat's sonar footprint (Vanderelst et al. 2010). The cross-section of each disc's surface can be described by a sine wave function whose peak amplitude represents the grating depth and whose number of periods per diameter defines the spatial frequency. Five spatial frequencies were tested, namely 4.4, 8.9, 17.8, 35.6, and 71.1 cycles/meter (Fig. 1.1B), corresponding to 2, 4, 8, 16 and 32 wave troughs per disc, respectively. From the bat's typical viewpoint in the setup, this corresponds to spatial frequencies of 0.125, 0.25, 0.5, 1 and 2 cycles/degree. Within each spatial frequency, a set of six grating depths was tested; namely ±16, ±8, ±4, ±2, ±1, and ±0 mm. The reference stimulus was a flat disc, identical to the ±0 mm control.

Experimental setup The experiments were performed inside a dark echo-attenuated chamber in a dark echo-attenuated room. Two infrared floodlights (TV6819, ABUS, Wetter, Germany) and a camera (WAT-902H2 Ultimate, Watec Co. LTD, Higashine, Japan) were mounted inside the chamber for observation. Both experimenter and control computer were stationed outside the chamber. Inside the chamber, a custom-built table held both the stimulus-presentation apparatus and the experimental cage (87 cm x 65 cm x 18 cm, W x H x D, built from wire mesh; Fig. 1.1C). Two stimulus discs (reference and test disc), were presented simultaneously. Both discs were placed on a carousel mechanism of the stimulus-presentation apparatus underneath the table, swivelled to their pseudo-randomly assigned positions and hoisted up into two holes in the tabletop. Due to this procedure, blinding was not possible. Infrared light barriers were fixed close to the bottom of both cage sidewalls to detect the presence (and thus decision) of the bat. They were connected to the serial port of a personal computer. Data recording and stimuli presentation order were controlled via a custom MATLAB R2007b application (The Mathworks, Inc., Natick, MA, USA).

General procedure Daily training sessions lasted 20-30 minutes per bat at 5 d per week, followed by a 2 d break. The experiment followed a two-alternative, forced-choice paradigm (2AFC) with food reinforcement. Once a bat sat at the starting position in the cage, both the flat disc (reference) and one grated disc (test) were presented. The position of the flat disc (left or right) was pseudo-random (Gellermann 1933). Bats had to move towards the flat disc, where they were rewarded with a mealworm as soon as they interrupted the corresponding light barrier. Then the bats returned to the starting position, where they waited in a closed starting box without sensory access to the outside until the discs were swivelled and a new trial started. Once a bat had learned this task (>70% correct choices on 5 consecutive days), data acquisition started for the same spatial frequency but with stepwise smaller grating depths, making the detection task more and more difficult. Daily data acquisition started with four consecutive trials presenting the largest grating depth of ±16 mm, continued with stepwise reduced grating depths, each presented for four trials. After a grating depth of ±0mm was reached, data acquisition returned to ±16 mm etc. until the daily session was completed. To keep the bats motivated, three or two trials instead of four trials per grating depth could be presented in a row, or easier trials (with larger grating depth) could be interspersed. Testing of one spatial frequency set was completed when 30 trials per grating depth and bat were recorded.

For the lowest tested spatial frequency of 0.125 cyc/deg, none of the bats reached the criterion level of 70% correct choices. To keep up motivation and to exclude the possibility that the bats had unlearned the task, trials with discs from a higher spatial frequency set were interspersed. In this manner, testing of all six grating depths at the 0.125 cyc/deg spatial frequency could be completed for two bats. For the other four bats, we needed to further adjust the procedure. We tested the detection of the spatial frequency of 0.125 cyc/deg only for the largest grating depth of ±16 mm and interspersed trials with the next higher spatial frequency of 0.25 cyc/deg at the largest grating depth of ±16mm (which the bats could detect). In this manner, we controlled for the possibility that lack of motivation or forgetting the task caused the bats to miss the criterion level at a spatial frequency of 0.125 cyc/deg. After none of the bats reached the criterion level at the largest grating depth at a spatial frequency of 0.125 cyc/deg, testing of the remaining smaller grating depths was dismissed and we conclude that bats cannot perceive ripples of ±16mm grating depth or smaller at a spatial frequency of 0.125 cyc/deg. Bat 6 dropped out due to pregnancy before completion of the 0.25 cyc/deg and 1 cyc/deg data sets.

Impulse responses and target strength measurements To evaluate the echo scenes reflected back by the experimental discs, we ensonified the discs with band-pass filtered white noise through a loudspeaker (Vifa Denmark A/S, Viborg, Denmark) and amplifier (AVR 445, Harman/Kardon, Stamford, CT, USA), and recorded the echoes with a ¼′′ measurement microphone and pre-amplifier (Type 40BF and 26CA, G.R.A.S. Sound & Vibration A/S, Holte, Denmark; protective grid removed) powered by a power module (G.R.A.S. Type 12AA).

Sound recording and noise playback were synchronized through an audio interface (Fireface 800, RME Audio AG, Haimhausen, Germany), which was controlled by SoundMexPro software (HörTech, Oldenburg, Germany) in MATLAB. The noise pass band ranged from 3 to 92 kHz. This encompasses the main frequency range used by P. discolor. Measurements were carried out with the speaker and microphone positioned at a distance of 40 cm to the disc at angles of 30°, 45°, 60° and 90° relative to the disc's center. We ensured a flat frequency response of the noise playback by filtering white noise with the speaker's compensatory impulse response (impulse response filter with cut-off frequencies of 3 and 92 kHz). Initial measurements of the speaker impulse response were conducted with the measurement microphone oriented perpendicular to the speaker at a distance of 40 cm.

We obtained each disc's acoustic impulse response (aIR) by cross-correlating the outgoing noise with the reflected echo in MATLAB (Version R2015a, The MathWorks Inc., Natick, MA, USA). aIRs and target strength depend on the ensonification angle. For the current comparison we chose target strengths at an ensonification angle of 45° relative to the disc's center, which matches the typical position of the bats during the experiment, and where target-strength differences were most conspicuous. Target strength differences between grated and flat discs were calculated by subtracting the root mean square of the corresponding impulse responses (test disc minus reference disc).

Data analysis Psychometric functions for five spatial frequencies between 0.125 cyc/deg and 2 cyc/deg were measured, i.e. the detection performance of the bat (in % correct detections over 30 trials) as a function of grating depth (from ±16 mm to ±0 mm). A sigmoid function was fitted to the psychometric function (MATLAB R2007b; The Mathworks, Inc., Natick, MA, USA) and the value of this fit at 70% was taken as threshold. In a 2AFC paradigm with 30 trials per point, 70% correct is significantly above chance level (95%-confidence intervals: 50.6-85.3%; Clopper-Pearson method for binomial confidence intervals). For the spatial frequency 0.125 cyc/deg, the threshold was assumed to be higher than ±16 mm, since none of the bats reached the significance level of 70%. Likewise, this was the case for Bat 5 at the spatial frequency 0.25 cyc/deg where it missed the 70% criterion. For calculating the average performance across bats, the threshold value in those conditions was conservatively set to ±16 mm.

CHAPTER 2

Flutter sensitivity in FM bats. Part I: delay modulation

Echolocating bats measure target distance by the time delay between call and echo. Target movement such as the flutter of insect wings induces delay modulations. Perception of delay modulations has been studied extensively in bats, but only concerning how well bats discriminate flutter frequencies, never with regard to flutter magnitude. We used an auditory virtual reality approach to generate changes in echo delay that were independent of call repetition rate, mimicking fluttering insect wings. We show that in the frequency-modulating (FM) bat *Phyllostomus discolor*, the sensitivity for modulations in echo delay depends on the rate of the modulation, with bats being most sensitive at modulation rates below 20 Hz and above 50 Hz. The very short duration of their calls compels FM bats to evaluate slow modulations (< about 100 Hz) across entire echo sequences. This makes them susceptible to interference between their own call repetition rate and the modulation rate. We propose that this phenomenon constitutes an echo-acoustic wagon-wheel effect. We further demonstrate how at high modulation rates, flutter sensitivity could be rescued by using spectral and temporal cues introduced by Doppler distortions. Thus, Doppler distortions may play a crucial role in flutter sensitivity in the hundreds of FM species worldwide.

A. Leonie Baier and Lutz Wiegrebe (2018) Flutter sensitivity in FM bats. Part I: delay modulation. *The Journal of Comparative Physiology A* (in press; https://doi.org/10.1007/s00359-018-1291-z) © The Authors 2018

Journal of Comparative Physiology A
https://doi.org/10.1007/s00359-018-1291-z

ORIGINAL PAPER

Flutter sensitivity in FM bats. Part I: delay modulation

A. Leonie Baier[1,2] · **Lutz Wiegrebe**[1]

Received: 22 June 2018 / Revised: 11 September 2018 / Accepted: 16 September 2018

Abstract

Echolocating bats measure target distance by the time delay between call and echo. Target movement such as the flutter of insect wings induces delay modulations. Perception of delay modulations has been studied extensively in bats, but only concerning how well bats discriminate flutter frequencies, never with regard to flutter magnitude. We used an auditory virtual reality approach to generate changes in echo delay that were independent of call repetition rate, mimicking fluttering insect wings. We show that in the frequency-modulating (FM) bat *Phyllostomus discolor*, the sensitivity for modulations in echo delay depends on the rate of the modulation, with bats being most sensitive at modulation rates below 20 Hz and above 50 Hz. The very short duration of their calls compels FM bats to evaluate slow modulations (< about 100 Hz) across entire echo sequences. This makes them susceptible to interference between their own call repetition rate and the modulation rate. We propose that this phenomenon constitutes an echo-acoustic wagon-wheel effect. We further demonstrate how at high modulation rates, flutter sensitivity could be rescued by using spectral and temporal cues introduced by Doppler distortions. Thus, Doppler distortions may play a crucial role in flutter sensitivity in the hundreds of FM species worldwide.

Keywords Biosonar · Echolocation · Virtual target · Doppler · Wagon-wheel effect

Abbreviations

CF	Constant frequency
FM	Frequency-modulating/frequency modulation
ICI	Inter-call interval
JND	Just noticeable difference
2AFC	Two-alternative forced choice

Introduction

Bats use echolocation to detect targets such as insect prey. They emit ultrasonic calls that are reflected off a target and return to the bat as echoes carrying information about the target. Two general types of echolocation calls have evolved in bats: frequency-modulated calls (FM calls) sweep through a broad band of frequencies within a few milliseconds, whereas constant-frequency calls (CF calls) keep a constant frequency over a much longer duration. The spatial acuity at which a target is localized increases with the range of frequencies an echolocation call covers, the call bandwidth. Acuity in target-distance assessment directly depends on bandwidth (Simmons 1973; Siemers and Schnitzler 2004), and acuity in azimuth and elevation indirectly depends on bandwidth, because broadband calls typically contain higher frequencies that give better spatial acuity due to shorter wavelength and higher directionality (Griffin 1958). The temporal resolution, at which changes in a target are depicted, however, is limited by the duration of the call (for, e.g., Doppler shift-based analyses) and/or call repetition rate (for time-domain analyses of echo-delay variation). A broadband, short FM call emitted at relatively low duty cycles therefore grants high spatial acuity at the possible expense of accuracy in detecting the movement of the target. Note that frequency-modulating bats (FM bats) constitute more than 80% of all echolocating species (Nowak 1994) and are able to navigate and forage in an environment full of moving targets.

Echolocation is a trinity of call, target and echo. Both call properties and target properties determine the properties

---

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00359-018-1291-z) contains supplementary material, which is available to authorized users.

✉ A. Leonie Baier
baier@orn.mpg.de

[1] Department Biology II, Ludwig Maximilians University Munich, Großhaderner Str. 2, 82152 Martinsried, Germany

[2] Acoustic and Functional Ecology Group, Max Planck Institute for Ornithology, Eberhard-Gwinner-Str. 11, 82319 Seewiesen, Germany

of the echo. Knowledge of the call properties and the echo properties in turn lets the bat draw conclusions about the target properties such as its location or surface structure (Simmons et al. 1974, 1983; Lawrence and Simmons 1982; Schmidt 1988b; Weissenbacher and Wiegrebe 2003; Grunwald et al. 2004; Holderied and von Helversen 2006; Firzlaff et al. 2007; Falk et al. 2011). For instance, the distance to the target is directly encoded in the time it takes the sound to travel from the bat to the target and back: the echo delay. Another parameter that changes with target distance is the amplitude of the returning echo. The further the sound travels, the fainter it becomes. Additionally, echo amplitude depends on the reflective strength of the target, the so-called target strength (Simmons et al. 2014). In other words, echo delay and echo amplitude co-vary with distance to the target, but echo delay is an absolute cue and echo amplitude is a relative cue for target distance.

Movement of the target itself can change its distance to the bat and its target strength. Thus, movement of the target introduces changes in echo delay and echo amplitude, which is referred to as echo-delay modulation and echo-amplitude modulation, respectively. For example, the fluttering wings of insects move back and forth, thereby changing the distance of the reflecting wing area. At the same time, the wings rotate, i.e., they change the size of the reflecting wing area, thereby changing the target strength (Griffin 1958; Roeder 1963). Consequently, periodic modulations in either echo delay or echo amplitude indicate fluttering insects. The ability to detect modulations of echo parameters is therefore often referred to as flutter sensitivity.

While there is a large body of literature regarding the basics of flutter sensitivity in CF bats (reviewed in Neuweiler 1990), flutter sensitivity in FM bats has been addressed by a mere handful of studies. The behavioral studies by Sum and Menne (1988), Roverud et al. (1991) and Grossetete and Moss (1998) have invited further inquiries. Flutter sensitivity was investigated only in terms of discriminating one flutter rate from another, not in terms of absolute sensitivity to the magnitude of the flutter, i.e., how large a flutter needs to be at a given flutter rate so that it can be detected by the bat. Moreover, neither study independently assessed bats' sensitivity to the two types of modulation introduced by the flutter: the modulation of echo delay and the modulation of echo amplitude. In both studies, the echolocating bat was presented with real targets, where echo delay and echo amplitude co-vary and it is therefore impossible to elucidate which information the bats extract from delay versus amplitude modulations. The key to solving this question is to create an auditory virtual reality for a bat and present virtual targets where amplitude and delay can be independently controlled.

A virtual target is communicated to the bat by a computer-generated echo played from a loudspeaker. Virtual targets produce simulated reflections, generated by picking up the bat's emission with an ultrasonic microphone, convolving it in real time with the acoustic impulse response of the virtual target, and playing back the result as an echo with a short latency of only a few milliseconds. The impulse response is the acoustic image of a target. It consists of the sum of all acoustic reflections of a target when it is ensonified with an acoustic impulse.

The classical phantom-target jitter experiments by Simmons (1979) that were repeated by Menne et al. (1989) took advantage of this method to selectively modulate only the echo delay and examine sensitivity to the magnitude of delay changes. However, these experiments were not designed to assess flutter sensitivity and therefore the modulation rate was not studied as an independent parameter: the rate of the rectangular echo-delay modulation was determined by the rate of sonar emissions; the phantom target 'jumped' back and forth with every emitted call. Notably, this is not an ecologically plausible modulation: in these experiments, target properties were adjusted according to the bat's vocal behavior, while in natural situations it is the reverse, i.e., bats adjust their ensonification behavior according to target properties (Moss and Surlykke 2010). Only one study has investigated bats' sensitivity to the magnitude of echo-delay modulation for a fixed, call-independent jitter frequency: Goerlitz et al. (2010) trained free-flying bats to discriminate between a stationary loudspeaker membrane and a membrane sinusoidally vibrating at 10 Hz. The perceived call-to-call jitter depended on call rate and call emission time in relation to the modulation phase. Thus, we hypothesize that for evaluation of changes across entire sequences of call–echo pairs the relation between call rate and modulation rate plays a crucial role.

To test this hypothesis, we combined both approaches, the sinusoidal modulation of either delay or amplitude independently from the bat's emission rate. This relies on virtual targets that change over time in just one of the two parameters, delay and amplitude. With modern processors that can operate in real time at high sampling rates, we can use time-variant impulse responses to create such virtual targets. The important advantage of a time-variant impulse response is that it truly simulates a moving target: it produces an echo with the target properties at the specific moment in time when the call is emitted, so that it interacts with call properties such as call duration and inter-call interval, affecting echo frequency, duration and delay. Like real moving targets, time-variant impulse responses can thus create, e.g., Doppler distortions and echo-amplitude modulations.

In this two-part study, we used a virtual environment to manipulate first only the modulation of delay at many call-independent modulation rates and second only the modulation of amplitude at many call-independent modulation rates. In this first of a series of two papers, we report on our investigations of the first part: bats' sensitivity to delay modulation. We demonstrate that sensitivity to echo-delay modulation strongly depends on modulation rate: bats show good sensitivity at low and high modulation rates and worse sensitivity for intermediate modulation rates around 20 and 50 Hz.

Materials and methods

Animals and permit

We used six adult male individuals of the neotropical omnivorous bat species *Phyllostomus discolor*, Wagner, 1843.These bats emit short (< 3 ms), downward frequency-modulated, multi-harmonic echolocation calls covering the frequency range between 45 and 100 kHz (Rother and Schmidt 1982). Bats were kept at the bat facilities in the Department Biology II of the Ludwig-Maximilians-University in Munich (12 h night/12 h day cycle, 65–75% relative humidity, 28 °C) with unlimited access to water at all times. On free days, the bats had ad libitum access to mixed fruit and mealworms (larval form of *Tenebrio molitor*) supplemented with oat, safflower oil, baby formula, minerals and vitamins (Vitakalk®). During training periods, the bats were with fed a pulp from fruit and supplementals in the experiment. All experiments complied with the principles of laboratory animal care and were conducted under the regulations of the current version of the German Law on Animal Protection (approval 55.2-1-54-2532-34-2015, Regierung von Oberbayern).

Experimental setup

Bats were trained to discriminate a rewarded stationary virtual target from an unrewarded target whose delay was sinusoidally modulated. The experiments were performed in a Y-maze inside a dark echo-attenuated chamber. The Y-maze consisted of a wire mesh floor, covered with removable cloth to clean the setup; the walls and ceiling of the maze were made of acoustically transparent gauze suspended between thin (about 2 mm diameter) metal rods at the corners of the maze. The starting area of the maze (lightly shaded area in Fig. 1) was about 10 cm wide and 15 cm long; each leg of the maze was also 10 cm wide and about 20 cm long. The inner height of the gauze was 12 cm throughout. As illustrated in Fig. 1, the loudspeakers and microphones were mounted directly behind the acoustically transparent gauze at the end of the two legs of the Y-maze. The experimenter was stationed outside the chamber and observed the experiment via an infrared camera (Abus® TV6819) and headphones. Stimulus presentation and data recording were controlled via a custom MatLab® R2007b application (The Mathworks, Inc., Natick, MA, USA).

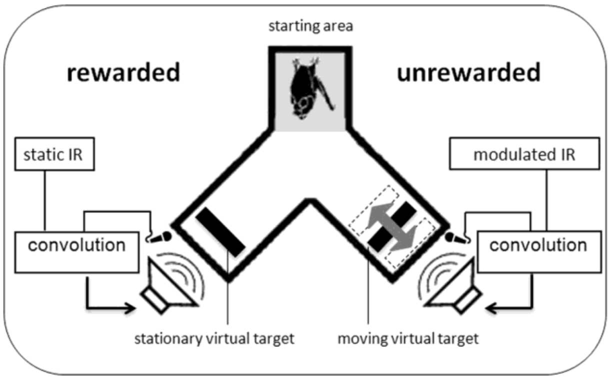

Fig. 1 Auditory virtual reality setup: six bats were trained to discriminate a virtual stationary target from a virtual target that simulated a periodic movement through modulating the echo delays at varying modulation rates ranging from 2 to 1000 Hz. All bats learned to indicate the pseudorandomly chosen position of the stationary target by crawling toward it from the depicted starting area after echolocating toward both targets. Virtual targets were created by convolving recorded echolocation calls in real time with a static or time-variant impulse response (IR)

Virtual-target production

During the full length of a trial, a bat would utter echolocation calls, and we implemented a time-variant delay between the call recording through the microphones and the virtual echo playback via the loudspeakers (henceforth, we will refer to the virtual echoes simply as echoes, although they were not echoes in the strict sense of an echo being a reflection from a physically present surface). Virtual targets were otherwise implemented as simple reflectors. Every change the bat chose to make in its emission sequence (e.g., change in call timing or call spectrum) was immediately reflected in the echoes. The only parameter that was systematically varied on our part was the echo delay.

Specifically, the bat's ultrasonic emissions were picked up by two microphones (SPM0204uD5, Knowles Corporation, Itasca, IL, USA) mounted 45° left and right relative to the bat's starting perch in a Y-maze. The microphone signals were amplified (octopre LE, Focusrite plc, Bucks, UK) and fed into the inputs of a real-time digital signal processor (260 kHz sampling rate; RX6, Tucker Davis Technologies, Gainesville, FL, USA). In the processor, a dynamic delay component, driven by a sine-wave generator of adjustable amplitude and frequency, was used in the modulated target's channel before the inputs were routed to the outputs, in addition to a constant base delay of 2500 µs in both channels.

Together with the AD, DA sampling delays of the RX6 and the physical delays from the bat to the microphone and from the speaker to the bat, the overall echo delay (without modulation) was about 4200 µs. This means that the virtual target was presented at a virtual distance of 72 cm to the emitting bat. It "appeared" outside the physical setup so that the bat could separate physical from virtual echoes more easily. Feedback suppression circuitry was included for safe operation. The outputs were connected via a stereo amplifier (Harman Kardon HK 6150; Harman Deutschland, Heilbronn, Germany) to two ultrasonic speakers (Technics EAS10TH800D; Panasonic Deutschland, Hamburg, Germany). Bats were tested with modulation depths of ± 2048 µs, ± 1024 µs, ± 512 µs, ± 128 µs, ± 64 µs, ± 32 µs, ± 16 µs and ± 8 µs for each of the following modulation rates: 2, 5, 10, 20, 50, 100, 200, 500, and 1000 Hz. At a modulation rate of 2 Hz, the signal undergoes one full modulation period within 500 ms, i.e., from original delay to shorter delay, to original delay, to longer delay, and finally to original delay again. At a modulation depth of ± 2048 µs, the virtual target moves within one modulation period from the reference distance of 72 cm (4200 µs) over a distance of 35 cm to the front, then again to the reference distance, then over a distance of 35 cm to the back, and finally back to the reference distance.

Behavioral procedure

Training/recording sessions (one to three per day) each lasted 10 min. Bats were trained on 5 days per week, followed by a 2-day break. The experiment followed a two-alternative, forced-choice paradigm (2AFC) with food reinforcement. Once a bat sat or perched in the starting area of the Y-maze, presentation of the virtual targets was switched on. The position of the stationary target (left or right) was pseudorandom (Gellermann 1933). Bats had to echolocate to find and move toward the stationary target, where they were rewarded as soon as they reached the corresponding feeder. Once a bat had learned this task (> 70% correct choices on 5 consecutive days), the modulation depth of the unrewarded target was reduced, making the discrimination task more difficult. During data acquisition, the modulation depth was then further reduced, starting with three consecutive trials presenting the highest modulation depth of ± 2048 µs, stepwise going down to three trials at a modulation depth of ± 8 µs and starting at ± 2048 µs again, etc. until the daily sessions were completed. To keep the bats motivated, easier trials (with a larger modulation depth) could be interspersed. Testing for one modulation rate set was completed when at least 30 trials were obtained per modulation depth and bat.

Behavioral data analysis

Percent correct performance of the animals as a function of modulation depth was fitted with a sigmoidal function and the value of this fit at 70% was taken as threshold (for $p < 0.05$ in a binomial test cf. Fig. 2). The threshold is the just-noticeable difference (JND) in modulation for a single bat at a single modulation rate.

Acoustic analyses

The echo properties depended both on the properties of the virtual targets themselves and critically on the properties of the emitted calls that the bats used to ensonify them. In our study, we manipulated the echo-acoustic target properties. We verified the echolocation-call properties with sound analysis. Additionally we verified the resulting echo properties generated by our delay-modulation hardware (the "virtual target machine") in response to an artificial echolocation call (see below). All acoustic analyses were done with custom MatLab® R2015a programs.

For sound analysis, the recorded call sequences were saved in a 3-s stereo ring buffer (192 kHz sampling rate, 24-bit resolution; Motu Ultralite, Motu, Cambridge, MA, USA) parallel to the virtual-target production. We high-pass filtered the stereo recordings at 35 kHz applying an eighth-order Butterworth filter. Then we extracted all echolocation calls above a fixed detection threshold (– 46 dB re. full scale) and with a minimum spacing of 5 ms between subsequent signals to exclude potentially recorded echoes. Temporal and spectral call parameters were taken from the channel with higher call level. We calculated the inter-call interval and the – 10 dB call duration. Call levels were calculated across a fixed 2.5 ms window centered on each call. The spectral centroid (weighted mean of frequencies present in the signal) was calculated from a time-averaged spectrogram with a 750 Hz binwidth. Minimum and maximum frequencies were extracted 10 dB below the peak frequency.

For the measurements of echo properties, we generated an artificial echolocation call as a multiharmonic FM-downward sweep of 1 ms duration with a fundamental frequency ranging from 23 to 19 kHz. This artificial echolocation call was then fed into the delay-modulation hardware (RX6; Tucker Davis) and manipulated in the same way as the real echolocation calls during the experiment. The signal at the processor's output was saved as the artificial echo, either from the stationary reflector or from the modulated reflector. For the latter, the outcome depended on the modulator phase that the sweep interacted with. We thus analyzed a coherent subset of echoes created at eight equally spaced phases in steps of 45° during the modulation.

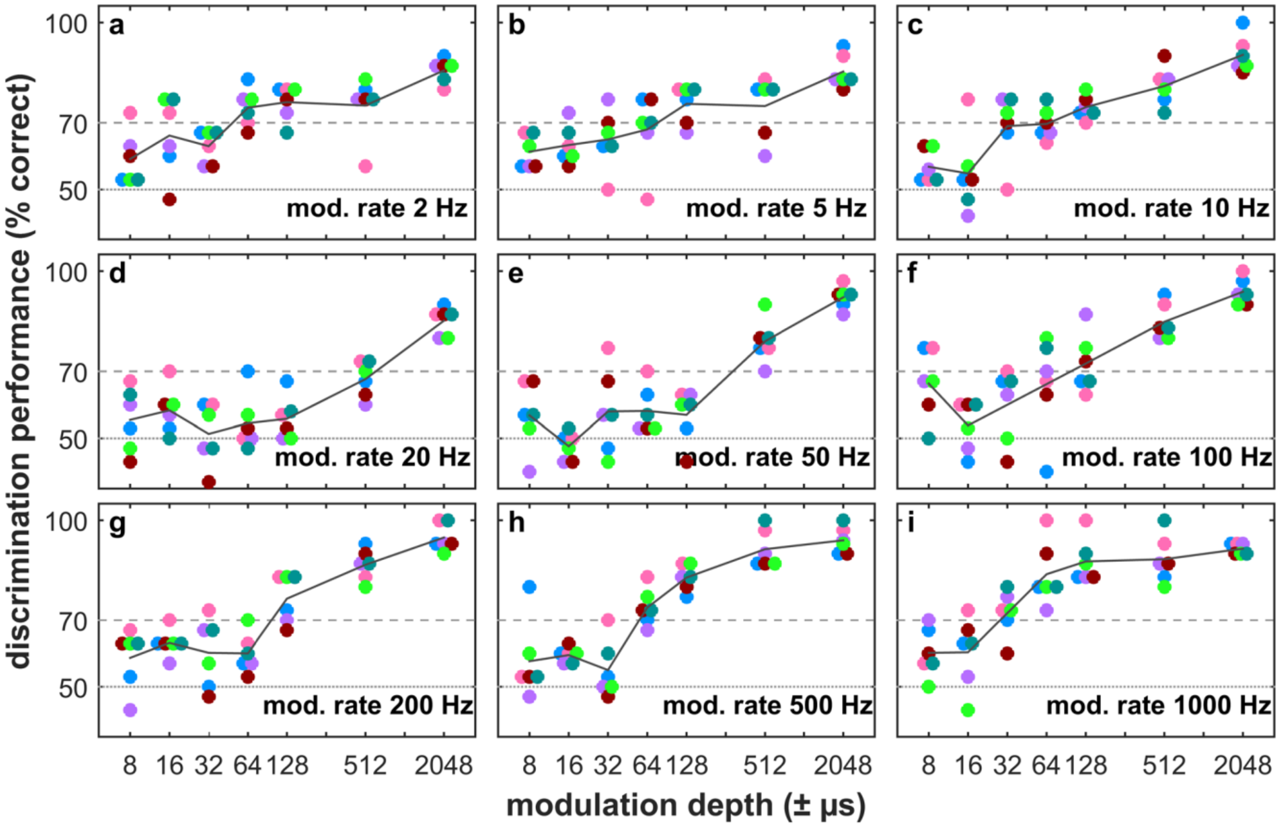

Fig. 2 Psychometric functions of echo-delay-modulation discrimination performance at nine modulation rates. Each colored dot marks one bat's discrimination performance across 30 trials. Black line plots depict the average discrimination performance. Horizontal dashed lines at 50 and 70% correct depict chance and significance level, respectively

Results

Behavioral response

Six male FM bats (*Phyllostomus discolor*) learned to discriminate between a virtual echo presented at a constant delay and a virtual echo presented at a modulated delay. We used the behavioral response of the bats to assess the just noticeable modulation depth, i.e., the threshold. For every bat, we extracted one threshold per modulation rate from the psychometric function to form a modulation transfer function across the nine modulation rates. It describes the sensitivity of the FM bat *P. discolor* for the modulation of echo delay.

Across all modulation rates, the results of all six bats confirmed our expectations for a psychometric function: discrimination was good at large echo-delay modulation depths and deteriorated with decreasing modulation depth (Fig. 2).

All bats faithfully (80–100% correct choices) discriminated a stationary target at a delay of 4200 µs from a target that oscillates in delay by ± 2048 µs around 4200 µs. On the contrary, the most difficult discrimination task we set, with a modulated target oscillating in delay by ± 8 µs around 4200 µs, could not be solved at all (40–67% correct choices for five of the nine tested modulation rates; Fig. 2b–e, g), or was only solved by one or two bats (70–80% correct choices for four modulation rates; Fig. 2a, f, h, i).

Discrimination performance systematically changed with the rate of the echo-delay modulation. Starting at the lowest modulation rate of 2 Hz, where the average discrimination threshold lies between ± 32 and ± 64 µs delay modulation, the bats' average performance deteriorates with increase in modulation rate up to a modulation rate of 20 Hz, where the average discrimination threshold lies between ± 512 and ± 2048 µs delay modulation. When the modulation rate is further increased up to 1000 Hz, bats' performance monotonically improves again (average discrimination threshold between ± 32 and ± 64 µs delay modulation).

The just-noticeable difference in modulation (JND) values extracted from the nine psychometric functions form the modulation transfer function that describes the bats' sensitivity for echo-delay modulation across nine modulation rates (Fig. 3). The modulation transfer function shows

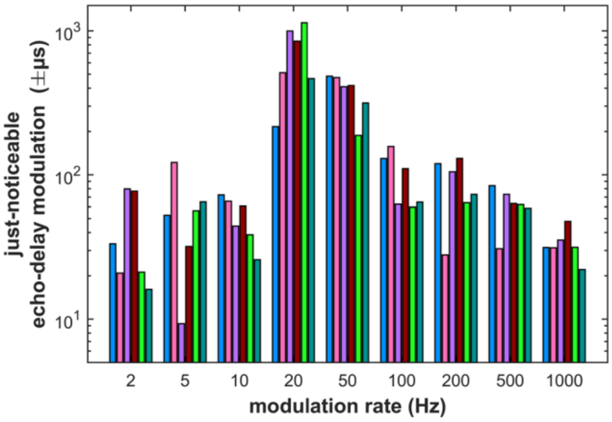

Fig. 3 Echo-delay modulation sensitivity. Detection thresholds are generally best at very low and very high modulation rates, and worst at intermediate modulation rates of 20 Hz and 50 Hz. Note the logarithmic ordinate. Bar colors represent individual bats' thresholds as extracted from sigmoidal fits to the psychometric functions in Fig. 2. Also note that modulation thresholds are given as peak values; they can be converted to peak-to-peak thresholds by multiplication with two

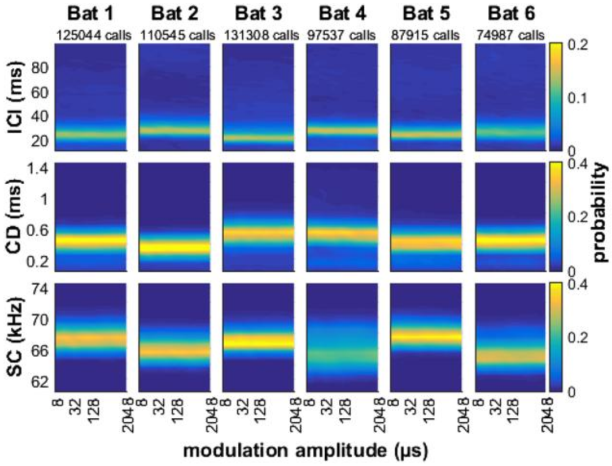

Fig. 4 Temporal and spectral properties of echolocation calls used by the bats for detecting echo-delay modulations with different modulation depths. The distribution of inter-call intervals (ICI, Row 1), call durations (CD, Row 2) and spectral centroids (SC, Row 3) did not change as a function of modulation depth (i.e., task difficulty) in either of the six bats (columns). Data are shown as normalized bin counts with color-coded probability

that the bats perform well at low and high modulation rates with echo-delay JNDs better than ± 100 µs. However, for intermediate modulation rates of 20 or 50 Hz, JNDs deteriorate and the bats need around ± 400 to ± 700 µs delay modulation to discriminate the stationary target from the modulated one.

Acoustic analyses

The bats' auditory percept depended not only on the echo-acoustic features of the virtual targets themselves, but critically on how the bats ensonified them. We performed acoustic analyses of the echolocation calls used by the bats during the behavioral experiment to better understand which sensory- and vocal-motor strategies the bats employed to solve the task. Additionally, we measured the echo properties generated by our delay-modulation hardware in response to an artificial echolocation call.

In the acoustic analysis of the echolocation calls, we first tested whether fundamental call parameters like inter-call intervals (ICIs), call duration, or the spectral centroid of the calls changed systematically when the task became more difficult for the bats, i.e., when the modulation depth decreased. The data show that, referenced against data from the highest modulation depth, the bats did not systematically modify any of these call parameters with increasing task difficulty (Fig. 4). Second, we determined whether these call parameters changed systematically with modulation rate. Here, we used only data from those trials where modulation depth was close to the perceptual threshold for this modulation rate and bat. The data show that ensonification parameters of the bats remain different across bats, but rather constant as a function of modulation rate (Fig. 5). In conclusion, we found no evidence for an adjustment of ensonification parameters, i.e., on the vocal-motor side of echolocation, that may serve to explain the dependence of echo-delay JNDs on modulation rate.

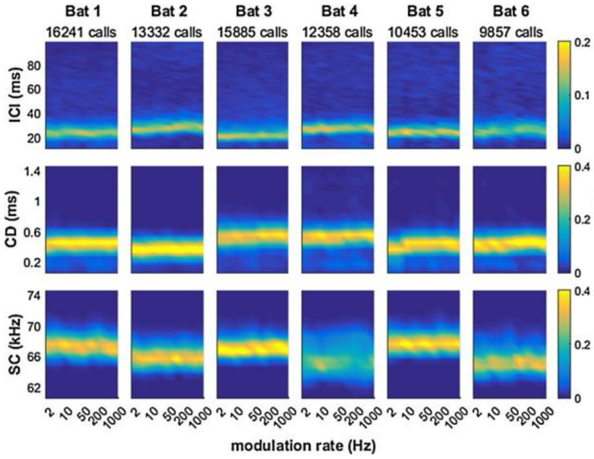

Fig. 5 Temporal and spectral properties of echolocation calls used by the bats for detecting echo-delay modulations with different modulation rates at a modulation depth that was just detectable for the bats. Again, the distribution of inter-call intervals (ICI, Row 1), call durations (CD, Row 2) and spectral centroids (SC, Row 3) did not change systematically as a function of presented modulation rate in the six bats. Again, data are shown as normalized bin counts with color-coded probability

During the analysis of the echolocation calls, it was conspicuous that the dominant ICI across all bats was around 20–40 ms (cf. first row of Figs. 4, 5). This typical ICI was the ICI used by the bats within call groups. The psychophysical results show that performance of the bats was worst around modulation rates of 20–50 Hz. This corresponded to modulation periods of 50 and 20 ms, respectively. We conclude that the bats performed worst when their call repetition rate was similar to the modulation rate and propose an echo-acoustic version of the visual wagon-wheel effect.

For the measurements of echo properties, we used a stereotyped *P. discolor* echolocation call, a 1 ms multiharmonic FM-downward sweep, and analyzed the artificial echoes as they were created by the delay-modulation hardware. We compared echo-power spectra and duration of echoes from the stationary reflector (black) and the modulated reflector (red), each for eight different modulator phases (Fig. 6). Delay-modulation depths had been adjusted such that the modulation was not detectable (± 8 µs), close to threshold (± 64 µs) or well above threshold (± 512 µs). With increasing modulation depth, the echo-delay modulation introduced Doppler-type distortions. The echoes from the modulated reflector differed from the stationary reflector's echo mainly in two ways: first, the frequency content was altered; second, the echo from the modulated reflector was either stretched or compressed in time relative to the echo from the stationary reflector. While these changes in echo spectrum and echo duration were moderate close to the threshold, distortions were dramatic for high modulation depths (± 512 µs). The range of frequencies below 35 kHz, which did not contain much energy in the echo from the stationary reflector, did contain energy in the echoes from the modulated reflector. Also, echo duration (numbers in panels) varied considerably between 0.64 and 1.96 ms (relative to the 1 ms call duration). Note that with the echo delay changing by ± 512 µs at a rate of 200 Hz, the virtual target would move back and forth at an average velocity of about 70 m/s, i.e., 250 km/h. These results illustrate that on top of the nominal perceptual cue, the time-variant echo delay, Doppler-type distortions may provide both spectral and temporal (echo duration) cues that may allow the bat to discriminate between stationary and time-variant reflectors.

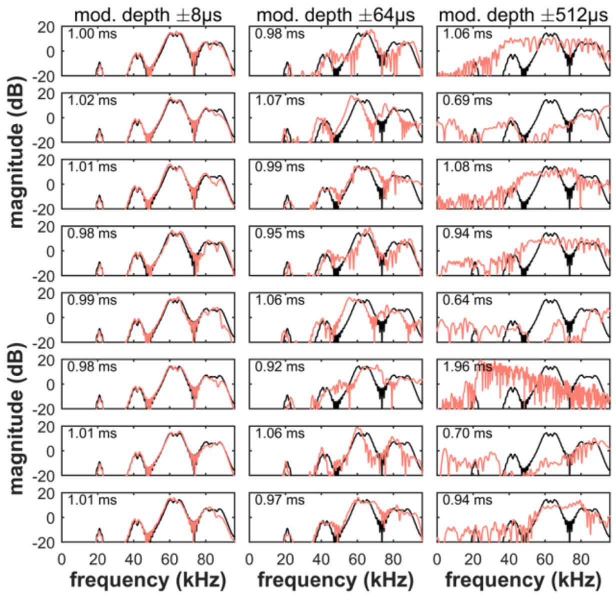

Fig. 6 Power spectra of echoes from eight different phases (rows) of a reflector that changes in echo delay at a rate of 200 Hz (red) in comparison to the echo from the stationary reflector (black). Depth of the echo-delay modulation is either non-detectable (± 8 µs, left column), close to threshold (± 64 µs, central column) or well above threshold (± 512 µs, right column). With increasing modulation depth, the differences between echoes from the modulated reflector (red) and the stationary reflector's echo (black) become more and more pronounced. In addition to the changes in the spectral properties, the Doppler-type distortions also introduce variation in the duration (numbers in panels) of the modulated reflector's echoes. The duration of the stationary echo is 1 ms

Discussion

When the distance between an echolocating bat and its target changes, the delay and amplitude of the echo change together, or co-vary. We found that the sensitivity of FM bats for modulations in echo delay depend on the rate of the modulation: *Phyllostomus discolor* bats were well able to distinguish a virtual target with constant echo delay from a virtual target whose echo delay was modulated over time when the modulation rates were either below 20 Hz or above 50 Hz. To the best of our knowledge, this study presents the first evidence of an echolocating FM bat detecting a delay modulation across a wide range of modulation rates that are independent of the bat's own emission rate.

In the following paragraphs, we first discuss delay-modulation sensitivity results in the context of previous work. Second, we address the bats' acoustic signals. Third, we propose an echo-acoustic wagon-wheel effect and discuss its origins and consequences. Fourth, we examine how fast target movements can induce alterations in both echo spectral composition and echo duration, which may serve as additional perceptual cues. Lastly, we discuss our results in an ecological context.

Delay-modulation sensitivity

In the following, we will compare the current results to those from both delay-discrimination and delay-jitter experiments. For convenience and comparability, we will convert all thresholds into peak-to-peak thresholds in microseconds. Our *P. discolor* bats were very sensitive to low and high modulation rates: for modulation rates below 20 Hz and above 50 Hz, we found delay-modulation thresholds between 66 and 194 µs (Fig. 3). These results are similar to the results from delay- (= range) discrimination experiments, which yielded thresholds between 36 and 176 µs (Simmons 1973; Roverud and Grinnell 1985; Surlykke and Miller 1985; Masters and Jacobs 1989; Miller 1991; Denzinger and Schnitzler 1994; Masters and Raver 1996).

At low modulation rates between 2 and 10 Hz, the current detection thresholds are also comparable to the delay-modulation thresholds of *Glossophaga soricina* FM bats: Goerlitz et al. (2010) measured delay-modulation thresholds of around 73 µs for large real targets moving at a 10 Hz modulation rate. At 10 Hz modulation rate, our bats on average detected a modulation of about 103 µs depth.

To intermediate modulation rates of 20 Hz and 50 Hz, our bats were much less sensitive, with thresholds around 1340 µs and 760 µs, respectively (Fig. 3). Heinrich and Wiegrebe (2013) showed that *P. discolor* bats can just discriminate stationary virtual targets when they differ in echo delay by around 250 µs. Current modulation thresholds are worse than 250 µs for modulation rates of 20 and 50 Hz but better than 250 µs for modulation rates above or below this range. This comparison indicates that our bats may have encountered special difficulties in detecting delay modulations for modulation rates around 20–50 Hz. This will be discussed in detail below.

The current thresholds and those we have compared them to so far (from delay-discrimination experiments) are worse than thresholds in delay-jitter experiments by orders of magnitude (Simmons 1979; Simmons et al. 1990, 2003, 2004; Menne et al. 1989; Moss and Schnitzler 1989). In those experiments, delay-jitter thresholds were always below 1 µs. It appears difficult to reconcile these diverging data sets. First, it is conceivable that this divergence results from the different bat species. However, delay-discrimination thresholds in *Eptesicus fuscus* were also much worse than the sub-microsecond thresholds reported in the jitter experiments in the same species (Denzinger and Schnitzler 1994, 1998). Second, the cause for the divergence may lie in the different virtual target stimulation: in the jitter experiments, delay was switched, in a quasi rectangular manner, after each emission of the bat. Thus the bat itself determined the average modulation rate, which is half the emission rate (and of course non-periodic due to the non-periodic emission patterns). In contrast, both (Goerlitz et al. 2010) and the current data were obtained with a sinusoidal modulator, completely independent of the bat's emission rate. Finally, differences between delay-jitter experiments on the one hand and delay-discrimination experiments on the other hand may lie in the fact that for the former, the bat can detect a change in delay at the same point in space (azimuth and elevation), while for the latter, the bat must compare delays across different azimuths. In summary, the current data remain hard to reconcile with the hyperacuity results in *E. fuscus*.

Acoustic analysis of echolocation calls

During target approach, bats systematically decrease both inter-call intervals (ICIs) and call duration to prevent the returning echo from overlapping with their next call (Griffin et al. 1960). For *P. discolor*, Linnenschmidt and Wiegrebe (2016) also observed that when a food source approached the bats, they systematically decreased the ICIs, the call duration and also the sound level of their emissions. In the current data, however, such an adjustment of call parameters is not seen (Figs. 4, 5): call parameters do not change, neither as a function of the delay-modulation depth (task difficulty) nor as a function of delay-modulation rate. Note that in the current experiments, there was no linear target motion, but target distance changed sinusoidally around a constant reference distance of 72 cm.

In the experiments of Linnenschmidt and Wiegrebe (2016), *P. discolor* use call durations of 0.4–0.7 ms and ICIs of 25–50 ms when echolocating toward a target at 70–80 cm distance. The call durations and ICIs we found in the current experiment (call duration around 0.4 ms and ICIs around 29 ms) are a good match given the here simulated distance of 72 cm between the virtual target and the bat. When we assume that perceived target distance dictates the call parameters that bats employ also in a stationary situation, we can explain why they neither adjusted their emissions to modulation depth nor to modulation rate. At the same time, this raises the question how this quasi-stable ICI interacts with the echo-delay modulation. In the following paragraph, we address our proposition that this represents an echo-acoustic version of the visual wagon-wheel effect.

Echo-acoustic wagon-wheel effect

We hypothesize that an interference of the ICI with the rate of the modulation generates an echo-acoustic wagon-wheel effect. In vision, the wagon-wheel effect is the result of temporal aliasing and describes the effect that under stroboscopic illumination a periodic movement may stay undetected because the illumination always occurs at the same phase of the movement. Transferred to the echo-acoustic system of bats, the unrewarded delay-modulated target may appear stationary (and thus more similar to the rewarded target) when the modulation rate of the modulated target equals the ensonification rate (= the reciprocal of the ICI) or an integer multiple thereof. Because the echoes perceived by the bat are the result of the given virtual target reflecting the echolocation call, the echo properties critically depend on the echolocation call parameters employed by the bats. We therefore would have expected the bats to adjust echolocation call parameters to task difficulty, i.e., to echo-delay modulation depth, but we found no evidence for such an adjustment (Fig. 4). Instead, the distribution of inter-call intervals (Fig. 4, Row 1), call durations (Row 2) and spectral centroids (Row 3) remained stable across modulation depths for individual bats. This finding supports the hypothesis that the bats very stereotypically adjust their ICI to target distance and do not intentionally vary the ICI to circumvent the wagon-wheel effect.

While we believe that this wagon-wheel effect may at least qualitatively explain the performance drop for intermediate modulation rates, this is not meant to suggest that the bats use the same perceptual cues to detect lower- and higher-rate modulations. At high modulation rates (corresponding to fast movements of the virtual target), other echo parameters may facilitate the psychophysical task (Beedholm and Møhl 1998). In the following paragraphs, we show that fast target movements can induce perceivable changes in both echo spectral composition and echo duration, related to Doppler distortions.

Doppler distortions

Doppler distortions arise from a sound being emitted or reflected by a moving object. In echolocation, an approaching target will produce an echo of a higher frequency than the emission; for a retreating target, the echo frequency is lower than the emission frequency. At first sight, Doppler distortions may be difficult to detect for FM bats, because the short duration and broad bandwidth of FM calls hamper the distortions' auditory detectability. Nevertheless, our measurements of Doppler distortions (Fig. 6) show that for higher modulation rates, Doppler distortions were prominent and perceptually relevant even at moderate modulation depths, comparable to the current perceptual thresholds. Specifically, the distortions caused the echo spectrum to spread below 35 kHz, where echoes from stationary targets were very faint. Such a pronounced difference in echo spectral composition is very likely perceived by bats (Schmidt 1988a; Weissenbacher and Wiegrebe 2003; Falk et al. 2011). Additionally, the distortions can almost halve or double the duration of the echo, depending on the modulator phase (0.64 ms and 1.96 ms relative to the 1.00 ms call duration). Again, this cue is well perceivable for bats (Schoernich and Wiegrebe 2008). Doppler distortions can even invert the spectro-temporal structure of the echolocation call. During an approach phase of a fast sinusoidal distance modulation, the downward frequency modulation of the emission can become an upward modulation in the echo.

The current results indicate that FM bats may be sensitive to Doppler distortions as generated by the wing beat of insects. It will be interesting to look in detail for the behavioral and neuronal mechanisms behind Doppler detection in terms of auditory temporal and tonotopic echo analysis. Specifically, analyses in Fig. 6 show that Doppler distortions affect both duration and spectral composition of echoes. Physiological sensitivity to echo spectral structure has been demonstrated both in *E. fuscus* (Sanderson and Simmons 2000) and *P. discolor* (Firzlaff et al. 2006; Borina et al. 2008; Heinrich et al. 2011). Also, neural sensitivity to echo duration was repeatedly demonstrated (Aubie et al. 2012; Fremouw et al. 2005). Finally, we have shown earlier that the *P. discolor* auditory cortex is quite sensitive to correlated changes in echo spectrum and duration and can even combine such features in a meaningful manner (Firzlaff et al. 2007).

Ecological relevance

Notably, we also presented our bats with target velocities that possibly exceed the ones found in fluttering insects (Vanderplank 1950). For instance, the modulation parameters exemplified in Fig. 6 were 200 Hz and ± 512 µs, corresponding to an average target velocity of 70 m/s. However, at the perceptual threshold for this modulation rate, the virtual target moves back and forth across a distance of about 15 mm within 5 ms. This results in an average velocity around 12 m/s. This lies well within the range of insect wing tip velocities (e.g., mosquito: 3 m/s, tsetse fly: 18 m/s; Vanderplank 1950).

We know little about the extent to which *P. discolor* hunts fluttering insects. In fact, it is often considered a mainly frugivorous species. However, its diet strongly depends on its geographic distribution and on season, ranging from almost pure nectarivory to almost pure insectivory (Kwiecinski 2006). The nitrogen isotopic composition of Mexican *P.discolor* is indistinguishable from that of carnivorous and sanguivorous animals (Schondube et al. 2001). While the stomach content of (Brazilian) *P. discolor* reportedly includes many insect species capable of flight (Willig et al. 1993), we cannot know whether this prey was caught in flight or gleaned off the substrate. Though we cannot finally conclude whether *P. discolor* itself could make use of a sensitive flutter detection system for prey detection, we assume that a true aerial hawking bat species would greatly benefit from flutter sensitivity in FM echolocation. Furthermore, flutter sensitivity would be advantageous for detecting other target movements that produce periodic echo-delay changes and thereby indirectly represent prey. For instance, advancing water ripples may indicate the presence of prey to the frog-eating bat, the Phyllostomid species *Trachops cirrhosus* (Halfwerk et al. 2014).

In conclusion, our work offers valuable insights into the perception of fluttering targets by FM bats. We have introduced a virtual reality approach with time-variant targets to assess sensitivity to echo-delay modulation. We demonstrated that in the FM bat *P. discolor*, the sensitivity for modulations in echo delay depends on the rate of the modulation. Sensitivity was best at modulation rates below 20 Hz and above 50 Hz. We suggest that an echo-acoustic wagon-wheel effect diminishes delay information when the modulation rate of the target matches bats' call repetition rate or an integer multiple thereof. We speculate that at high modulation rates, bats instead use spectral and temporal cues introduced by Doppler distortions.

The use of virtual targets allows the clean segregation of echo-delay and echo-amplitude modulations for flutter detection. The following paper will address bats' sensitivity to echo-amplitude modulations. We will show that echo-amplitude modulation is perceived quite differently from echo-delay modulation, indicating fundamentally different neural processing of these co-occurring echo features.

Acknowledgements Open access funding provided by Max Planck Society. We thank H. Goerlitz, H. Peremans, F. Rosales, and the Wiegrebe lab for discussion and gratefully acknowledge the two anonymous referees for their detailed comments on the manuscript. We are grateful to E. Lattenkamp and M. Linnenschmidt for help during data acquisition. We thank B. Grothe for providing excellent research infrastructure. All experiments complied with the principles of laboratory animal care and were conducted under the regulations of the current version of the German Law on Animal Protection (approval 55.2-1-54-2532-34-2015, Regierung von Oberbayern).

Author contributions ALB and LW conceived and designed the study. ALB performed the experiment. ALB and LW analyzed the data, and wrote and revised the manuscript.

Funding This work was supported by the Deutsche Forschungsgemeinschaft (DFG WI 1518/13-1 to LW). ALB was supported by the International Max Planck Research School for Organismal Biology.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Aubie B, Sayegh R, Faure PA (2012) Duration tuning across vertebrates. J Neurosci 32(18):6373–6390

Beedholm K, Møhl B (1998) Bat sonar: an alternative interpretation of the 10-ns jitter result. J Comp Physiol A 182(3):259–266. https://doi.org/10.1007/s003590050176

Borina F, Firzlaff U, Schuller G, Wiegrebe L (2008) Representation of echo roughness and its relationship to amplitude-modulation processing in the bat auditory midbrain. Eur J Neurosci 27(10):2724–2732. https://doi.org/10.1111/j.1460-9568.2008.06247.x

Denzinger A, Schnitzler H-U (1994) Echo SPL influences the ranging performance of the big brown bat, *Eptesicus fuscus*. J Comp Physiol A 175(5):563–571. https://doi.org/10.1007/bf00199477

Denzinger A, Schnitzler H-U (1998) Echo SPL, training experience, and experimental procedure influence the ranging performance in the big brown bat, Eptesicus fuscus. J Comp Physiol A 183(2):213–224. https://doi.org/10.1007/s003590050249

Falk B, Williams T, Aytekin M, Moss CF (2011) Adaptive behavior for texture discrimination by the free-flying big brown bat, *Eptesicus fuscus*. J Comp Physiol A 197(5):491–503. https://doi.org/10.1007/s00359-010-0621-6

Firzlaff U, Schörnich S, Hoffmann S, Schuller G, Wiegrebe L (2006) A neural correlate of stochastic echo imaging. J Neurosci 26(3):785–791

Firzlaff U, Schuchmann M, Grunwald JE, Schuller G, Wiegrebe L (2007) Object-oriented echo perception and cortical representation in echolocating bats. PLoS Biol 5(5):e100

Fremouw T, Faure PA, Casseday JH, Covey E (2005) Duration selectivity of neurons in the inferior colliculus of the big brown bat: tolerance to changes in sound level. J Neurophysiol 94(3):1869–1878

Gellermann LW (1933) Chance orders of alternating stimuli in visual discrimination experiments. Ped Sem J Gen Psychol 42(1):206–208

Goerlitz HR, Geberl C, Wiegrebe L (2010) Sonar detection of jittering real targets in a free-flying bat. J Acoust Soc Am 128(3):1467–1475. https://doi.org/10.1121/1.3445784

Griffin DR (1958) Listening in the dark: the acoustic orientation of bats and men. Yale University Press, Oxford

Griffin DR, Webster FA, Michael CR (1960) The echolocation of flying insects by bats. Anim Behav 8(3):141–154. https://doi.org/10.1016/0003-3472(60)90022-1

Grossetete A, Moss CF (1998) Target flutter rate discrimination by bats using frequency-modulated sonar sounds: behavior and signal processing models. J Acoust Soc Am 103(4):2167–2176

Grunwald JE, Schornich S, Wiegrebe L (2004) Classification of natural textures in echolocation. Proc Natl Acad Sci USA 101(15):5670–5674

Halfwerk W, Jones PL, Taylor RC, Ryan MJ, Page RA (2014) Risky ripples allow bats and frogs to eavesdrop on a multisensory sexual display. Science 343(6169):413–416. https://doi.org/10.1126/science.1244812

Heinrich M, Wiegrebe L (2013) Size constancy in bat biosonar? Perceptual interaction of object aperture and distance. PLoS One 8(4):e61577. https://doi.org/10.1371/journal.pone.0061577

Heinrich M, Warmbold A, Hoffmann S, Firzlaff U, Wiegrebe L (2011) The sonar aperture and its neural representation in bats. J Neurosci 31(43):15618–15627. https://doi.org/10.1523/jneurosci.2600-11.2011

Holderied MW, von Helversen O (2006) Binaural echo disparity'as a potential indicator of object orientation and cue for object recognition in echolocating nectar-feeding bats. J Exp Biol 209(17):3457–3468

Kwiecinski GG (2006) *Phyllostomus discolor*. Mamm Species 801:1–11

Lawrence BD, Simmons JA (1982) Echolocation in bats—the external ear and perception of the vertical positions of targets. Science 218(4571):481–483

Linnenschmidt M, Wiegrebe L (2016) Sonar beam dynamics in leaf-nosed bats. Sci Rep 6:29222. https://doi.org/10.1038/srep29222

Masters WM, Jacobs SC (1989) Target detection and range resolution by the big brown bat (*Eptesicus fuscus*) using normal and time-reversed model echoes. J Comp Physiol A 166(1):65–73. https://doi.org/10.1007/bf00190211

Masters W, Raver K (1996) The degradation of distance discrimination in big brown bats (*Eptesicus fuscus*) caused by different interference signals. J Comp Physiol A 179(5):703–713

Menne D, Kaipf I, Wagner I, Ostwald J, Schnitzler HU (1989) Range estimation by echolocation in the bat *Eptesicus fuscus*: trading of phase versus time cues. J Acoust Soc Am 85(6):2642–2650. https://doi.org/10.1121/1.397758

Miller LA (1991) Arctiid moth clicks can degrade the accuracy of range difference discrimination in echolocating big brown bats, *Eptesicus fuscus*. J Comp Physiol A 168(5):571–579. https://doi.org/10.1007/bf00215079

Moss CF, Schnitzler H-U (1989) Accuracy of target ranging in echolocating bats: acoustic information processing. J Comp Physiol A 165(3):383–393

Moss CF, Surlykke A (2010) Probing the natural scene by echolocation in bats. Front Behav Neurosci. https://doi.org/10.3389/fnbeh.2010.00033

Neuweiler G (1990) Auditory adaptations for prey capture in echolocating bats. Physiol Rev 70(3):615–641

Nowak RM (1994) Walker's bats of the world. JHU Press, Baltimore

Roeder KD (1963) Echoes of ultrasonic pulses from flying moths. Biol Bull 124(2):200–210. https://doi.org/10.2307/1539496

Rother G, Schmidt U (1982) The influence of visual information on echolocation in *Phyllostomus discolor* (Chiroptera). Z Säugetierkunde 47(6):324–334

Roverud RC, Grinnell AD (1985) Discrimination performance and echolocation signal integration requirements for target detection and distance determination in the CF/FM bat, *Noctilio albiventris*. J Comp Physiol A 156(4):447–456

Roverud RC, Nitsche V, Neuweiler G (1991) Discrimination of wingbeat motion by bats, correlated with echolocation sound pattern. J Comp Physiol A 168(2):259–263

Sanderson MI, Simmons JA (2000) Neural responses to overlapping FM sounds in the inferior colliculus of echolocating bats. J Neurophysiol 83(4):1840–1855

Schmidt S (1988a) Discrimination of target surface structure in the echolocating bat, *Megaderma lyra*. In: Nachtigall PE, Moore PWB (eds) Animal sonar: processes and performance. Springer, Boston, pp 507–511. https://doi.org/10.1007/978-1-4684-7493-0_52

Schmidt S (1988b) Evidence for a spectral basis of texture perception in bat sonar. Nature 331(6157):617

Schoernich S, Wiegrebe L (2008) Phase sensitivity in bat sonar revisited. J Comp Physiol A 194(1):61–67. https://doi.org/10.1007/s00359-007-0290-2

Schondube JE, Herrera-M LG, Martínez del Rio C (2001) Diet and the evolution of digestion and renal function in phyllostomid bats. Zoology 104(1):59–73. https://doi.org/10.1078/0944-2006-00007

Siemers BM, Schnitzler HU (2004) Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. Nature 429(6992):657–661

Simmons JA (1973) The resolution of target range by echolocating bats. J Acoust Soc Am 54(1):157–173. https://doi.org/10.1121/1.1913559

Simmons JA (1979) Perception of echo phase information in bat biosonar. Science 204(4399):1336–1338. https://doi.org/10.1126/science.451543

Simmons J, Lavender W, Lavender B, Doroshow C, Kiefer S, Livingston R, Scallet A, Crowley D (1974) Target structure and echo spectral discrimination by echolocating bats. Science 186(4169):1130–1132

Simmons J, Kick S, Lawrence B, Hale C, Bard C, Escudie B (1983) Acuity of horizontal angle discrimination by the echolocating bat, *Eptesicus fuscus*. J Comp Physiol A 153(3):321–330

Simmons JA, Ferragamo M, Moss CF, Stevenson SB, Altes RA (1990) Discrimination of jittered sonar echoes by the echolocating bat, *Eptesicus fuscus*—the shape of target images in echolocation. J Comp Physiol A 167(5):589–616

Simmons JA, Ferragamo MJ, Sanderson MI (2003) Echo delay versus spectral cues for temporal hyperacuity in the big brown bat, *Eptesicus fuscus*. J Comp Physiol A 189(9):693–702. https://doi.org/10.1007/s00359-003-0444-9

Simmons JA, Neretti N, Intrator N, Altes RA, Ferragamo MJ, Sanderson MI (2004) Delay accuracy in bat sonar is related to the reciprocal of normalized echo bandwidth, or Q. Proc Natl Acad Sci USA 101(10):3638–3643

Simmons JA, Houser D, Kloepper L (2014) Localization and classification of targets by echolocating bats and dolphins. In: Surlykke A, Nachtigall PE, Fay RR, Popper AN (eds) Biosonar. Springer, New York, pp 169–193. https://doi.org/10.1007/978-1-4614-9146-0_6

Sum YW, Menne D (1988) Discrimination of fluttering targets by the FM-bat *Pipistrellus stenopterus*. J Comp Physiol A 163(3):349–354. https://doi.org/10.1007/bf00604010

Surlykke A, Miller LA (1985) The influence of arctiid moth clicks on bat echolocation; jamming or warning? J Comp Physiol A 156(6):831–843. https://doi.org/10.1007/bf00610835

Vanderplank FL (1950) Air-speed/wing-tip speed ratios of insect flight. Nature 165:806. https://doi.org/10.1038/165806a0

Weissenbacher P, Wiegrebe L (2003) Classification of virtual objects in the echolocating bat, *Megaderma lyra*. Behav Neurosci 117(4):833–839. https://doi.org/10.1037/0735-7044.117.4.833

Willig MR, Camilo GR, Noble SJ (1993) Dietary overlap in frugivorous and insectivorous bats from edaphic cerrado habitats of Brazil. J Mammal 74(1):117–128

Flutter sensitivity in FM bats. Part II: amplitude modulation

Bats use echolocation to detect targets such as insect prey. The echolocation call of frequency-modulating bats (FM bats) typically sweeps through a broad range of frequencies within a few milliseconds. The large bandwidth grants the bat high spatial acuity in depicting the target. However, the extremely short call duration and the overall low duty cycle of call emission impair the bat's capability to detect e.g. target movement. Nonetheless, FM bats constitute more than 80% of all echolocating species and are able to navigate and forage in an environment full of moving targets. We used an auditory virtual reality approach to generate changes in echo amplitude reflective of fluttering insect wings independently from other confounding parameters. We show that the FM bat *Phyllostomus discolor* successfully detected these modulations in echo amplitude and that their performance increased with the rate of the modulation, mimicking faster insect wing-beats. The ability of FM bats to detect amplitude modulations of echoes suggests a release from the trade-off between spatial and temporal acuity and highlights the diversity of selective pressures working on the echolocation system of bats.

A. Leonie Baier, Kristin-Jasmin Stelzer, and Lutz Wiegrebe (2018) Flutter sensitivity in FM bats. Part II: amplitude modulation. *The Journal of Comparative Physiology A* (in press; https://doi.org/10.1007/s00359-018-1292-y) © The Authors 2018

Journal of Comparative Physiology A
https://doi.org/10.1007/s00359-018-1292-y

ORIGINAL PAPER

Flutter sensitivity in FM bats. Part II: amplitude modulation

A. Leonie Baier[1,2] · **Kristin-Jasmin Stelzer**[1] · **Lutz Wiegrebe**[1]

Received: 22 June 2018 / Revised: 12 September 2018 / Accepted: 16 September 2018

Abstract
Bats use echolocation to detect targets such as insect prey. The echolocation call of frequency-modulating bats (FM bats) typically sweeps through a broad range of frequencies within a few milliseconds. The large bandwidth grants the bat high spatial acuity in depicting the target. However, the extremely short call duration and the overall low duty cycle of call emission impair the bat's capability to detect e.g. target movement. Nonetheless, FM bats constitute more than 80% of all echolocating species and are able to navigate and forage in an environment full of moving targets. We used an auditory virtual reality approach to generate changes in echo amplitude reflective of fluttering insect wings independently from other confounding parameters. We show that the FM bat *Phyllostomus discolor* successfully detected these modulations in echo amplitude and that their performance increased with the rate of the modulation, mimicking faster insect wing-beats. The ability of FM bats to detect amplitude modulations of echoes suggests a release from the trade-off between spatial and temporal acuity and highlights the diversity of selective pressures working on the echolocation system of bats.

Keywords Biosonar · Echolocation · SAM · Virtual target · Wagon-wheel effect

Abbreviations

AM	Amplitude modulation
CF	Constant frequency
FM	Frequency modulating/frequency modulation
ICI	Inter-call interval
SAM	Sinusoidal amplitude modulation
2AFC	Two-alternative forced choice

Introduction

Bats emit ultrasonic calls and perceive targets such as insect prey by extracting information from the returning echoes. The short, broadband echolocation signals of frequency-modulating bats (FM bats) are very well suited to describe a three-dimensional static layout, due to their similarity to a Dirac Impulse (an impulse with infinitely broad bandwidth and infinitesimally short duration). As already argued in the first paper of this series (Baier and Wiegrebe this issue) though, short broadband calls emitted at low-duty-cycle (Fenton et al. 2012) are not well suited to describe a time-variant system, that is, movement of the ensonified target(s) and/or the bat itself. A frequency-modulated call (FM call) therefore grants high spatial acuity at the expense of accuracy in detecting the movement of the target. Nevertheless, more than 80% of echolocating bat species use FM calls (Nowak 1994).

The fluttering wings of edible insect prey generate a modulation of both echo delay and echo amplitude over time (Neuweiler 1984; Schnitzler et al. 1985). Therefore, bats' ability to detect modulations of echo parameters is often referred to as flutter sensitivity. In our two-part study we used a virtual environment to investigate the two aspects of flutter sensitivity in bats: first, sensitivity to the modulation of echo delay and second, sensitivity to the modulation of echo amplitude.

In the first paper of the series (Baier and Wiegrebe this issue), we showed that despite the low-duty-cycle echolocation of the FM bat under study (*Phyllostomus discolor*) these bats can detect echo-delay modulations even as fast as 1000 Hz, which corresponds to the fastest wing beat rate found in insects (Sotavalta 1953). We demonstrated good sensitivity to modulations up to 10 Hz as well, but markedly worse sensitivity for an intermediate modulation

---

✉ A. Leonie Baier
baier@orn.mpg.de

[1] Department Biology II, Ludwig Maximilians University Munich, Großhaderner Str. 2, 82152 Martinsried, Germany

[2] Acoustic and Functional Ecology Group, Max Planck Institute for Ornithology, Eberhard-Gwinner-Str. 11, 82319 Seewiesen, Germany

range between 20 and 50 Hz. We discussed these results with respect to the different perceptual cues (nominal delay cues for slow modulations and Doppler distortions for fast modulations). We further proposed an echo-acoustic 'wagon wheel effect', i.e. an interaction of the modulation rate with the repetition rate of the bats' emissions. Conspicuously the bats were worse at detecting echo-delay modulations when the modulation rate was in the same range as the call emission rate [within strobe groups of echolocation calls as they are typically emitted by our bats and many other FM bat species (Moss and Surlykke 2010)].

If the reason for the worse detection of intermediate delay-modulation rates lies in the wagon-wheel effect, one might predict that this would equally apply to the detection of the other echo feature that is periodically modulated by insect wing beats, namely the echo amplitude or target strength. Notably, echo delay and echo amplitude are encoded differently in the bat ascending auditory pathway (Hagemann et al. 2010; Hechavarría et al. 2013; Greiter and Firzlaff 2017; Measor et al. 2018). These considerations guided us in this second part of our two-part study where we explicitly address the question how sensitive the bat *P. discolor* is for modulations of echo amplitude over time.

To answer this question we used the auditory-virtual-reality setup that we already employed in the companion study (Baier and Wiegrebe this issue). The real-time digital signal processing allowed us to manipulate the modulation of amplitude at several modulation rates while leaving echo delay constant. We demonstrate that echo-acoustic sensitivity to echo-amplitude modulation did follow a different trajectory with modulation rate in comparison to sensitivity to echo-delay modulation. Further, we illustrate that at high modulation rates, spectral cues likely dominated the bats' performance but that these spectral cues are not Doppler distortions.

Materials and methods

For our two-part study we designed two behavioral experiments to manipulate first only the modulation of delay (Baier and Wiegrebe this issue) and second only the modulation of amplitude (current study). Here we used the same experimental animals and paradigm as in part one. For a detailed description of the methods, see therefore Baier and Wiegrebe, this issue.

Animals and permit

We used four adult male individuals of the bat species *Phyllostomus discolor*, Wagner, 1843. All experiments were conducted under the regulations of the German Law on Animal Protection (approval 55.2-1-54-2532-34-2015, Regierung von Oberbayern).

Virtual-target production

During the full length of a trial, a bat would utter echolocation calls. We recorded the emitted calls, implemented a time-variant amplitude modification and broadcasted the resulting virtual echoes via the loudspeakers, all in real-time (Fig. 1). Henceforth we will refer to the virtual echoes simply as echoes, although they were not echoes in the strict sense of an echo being a reflection from a physically present surface. Besides the modified echo amplitude, virtual targets were otherwise implemented as simple reflectors. Every change the bat chose to make in its emission sequence (e.g. change in call timing or call spectrum) was immediately reflected in the echoes. The only parameter that was systematically varied on our part was the echo amplitude (target strength).

We used two impulse responses representing two virtual targets: The target strength of one target was kept constant while the target strength of the other target was modulated over time, i.e., the amplitude of one echo was constant while the other was modulated. We tested the sensitivity of the bats to amplitude modulation at six different modulation rates: 5, 10, 20, 50, 200, and 500 Hz. At a modulation rate of 5 Hz, the signal undergoes one full modulation period within 200 ms, i.e., from original loudness to amplification, to original loudness, to attenuation, and finally to original loudness again. We tested modulation depths decreasing in steps of 2 dB, starting from 0 dB until a particular bat's threshold was found (see "Behavioral procedure" section). The modulation depth of 0 dB corresponds to 100% modulation (easiest condition), i.e. the amplitude oscillates between twice the linear amplitude of the unmodulated target and silence. The modulation depth of – 26 dB corresponds to 5% modulation, i.e. the linear amplitude oscillates only between 105% and 95% of the linear amplitude of the unmodulated target (most difficult condition; Fig. 1b).

Behavioral procedure

We quantified sensitivity for the modulation of echo amplitude with a formal psychophysical experiment, following a two-alternative, forced-choice (2AFC) paradigm. Bats were offered a choice between the constant and the modulated target and were trained to choose the constant target for a food reward. A bat indicated its choice by crawling down one arm of a *y*-shaped maze towards the target and the corresponding feeder (Fig. 1a). For each bat we recorded a full psychometric function per modulation rate. A psychometric function describes the relationship between the stimulus magnitude and the subject's response. Here we looked at

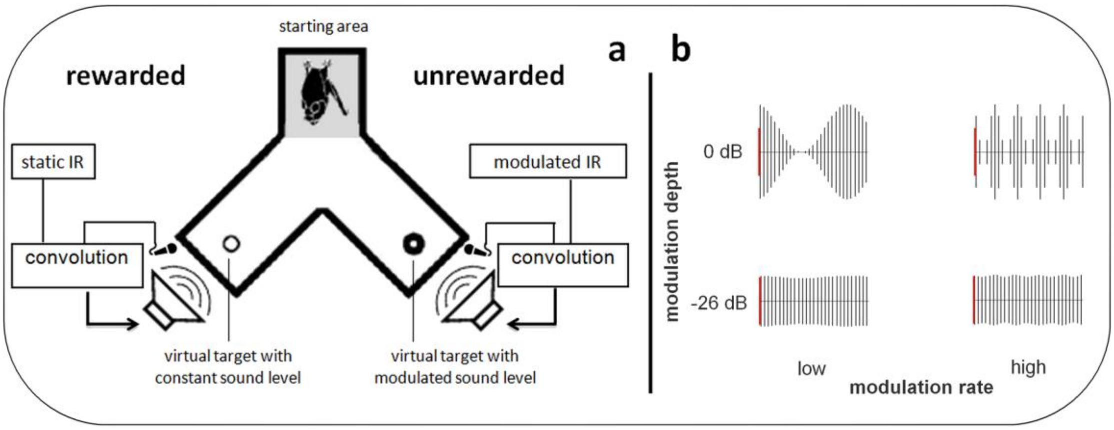

Fig. 1 Auditory virtual reality setup. **a** Four bats were trained to discriminate a virtual target with constant sound level from a virtual target whose sound level was modulated at varying modulation rates ranging from 5 to 500 Hz. All bats learned to indicate the pseudorandomly chosen position of the static target by crawling toward it from the depicted starting area after echolocating toward both targets. Virtual targets were created by convolving recorded echolocation calls in real time with a static or time variant impulse response (IR). **b** The depth of the modulation ranged from 0 dB (corresponding to the sound level oscillating between silence and 200% of the input sound level) to − 26 dB (corresponding to the sound level oscillating between 95 and 105% of the input sound level). Red bars indicate 100% sound level. Black lines symbolize single echoes. Note that for visualization purposes call repetition rate is not to scale, but set about four times as high as we found in our bats

the relationship between the depth of the modulation and the bat's performance on the discrimination of constant vs. modulated target. Discrimination performance was measured in percent correct choices over a minimum of 30 trials per modulation depth. We tested modulation depths ranging from 0 to − 26 dB in steps of 2 dB. For each bat, data collection was complete on reaching a modulation depth below threshold, i.e., where performance dropped below 70% for two consecutive modulation depths. Each completed psychometric function consisted of performance measures at at least seven different modulation depths (≥ 210 trials). The threshold extracted from the psychometric function is the just-noticeable difference (JND) in modulation for the respective bat at the respective modulation rate. We fitted the psychometric function with a sigmoidal function and estimated the threshold for the respective animal and modulation rate from the fit's value at 70% correct performance ($p < 0.05$, binomial test). The entirety of the extracted threshold values described the sensitivity of *P. discolor* for the modulation of echo amplitude across a wide range of modulation rates.

Acoustic analyses

The echo properties depended both on the properties of the virtual targets themselves and critically on the properties of the emitted calls that the bats used to ensonify them. In our study, we manipulated the echo-acoustic target properties. We verified the echolocation-call properties with sound analysis. We simulated the resulting echo properties with regard to the nominal perceptive cue (amplitude modulation) and to additional perceptive cues that may serve the bats to detect the echo-amplitude modulations.

For sound analysis, the recorded call sequences were saved parallel to the virtual-target production. After high-pass filtering the stereo recordings at 35 kHz (8th order butterworth filter), we extracted echolocation calls and calculated temporal and spectral call parameters from the channel with higher call level (inter-call interval and-10 dB call duration; spectral centroid, i.e., weighted mean of the frequencies present in the signal, based on time-averaged spectrogram with a 750 Hz binwidth). Due to technical problems we were forced to replace the original audio-interface (Motu Ultralite, Motu, Cambridge, MA, USA) used to record echolocation calls during ongoing data acquisition. The new interface's (RME Fireface 400, Audio AG, Haimhausen, Germany) recording properties introduced a downward shift in the frequency content of recorded calls compared to older recordings.

For the simulation of perceived echo cues we recreated the virtual target's time-variant impulse response (i.e. the modulator) as a sine wave and multiplied it with either recorded echolocation-call sequences or artificial echolocation calls (multiharmonic FM-downward sweep of 1 ms duration with fundamental frequency ranging from 23 to 19 kHz). First, we looked at the nominal cue, i.e. at the amplitude differences between two consecutive echolocation calls. Due to the time-variant nature of the impulse response, there were no two fixed echo amplitudes that constituted the difference, but a range of potential amplitudes, depending on both the bats' timing of echolocation call emission and the phase at which the emitted calls hit the modulator. We used eight different modulator phases to simulate a comprehensive subset of possible call-to-call amplitude differences. The amplitude difference between two consecutive echoes naturally also depends on the inter-call interval (ICI). For our simulations we used real ICIs extracted during sound analysis. Second, we addressed potential additional cues that might arise from the interaction of one single echolocation call with the modulator. Again, the outcome depends on the modulator phase that the call interacts with. We thus simulated a subset of possible interactions by multiplying one artificial call with eight different modulator phases.

Results

Behavioral response

Four male FM bats (*Phyllostomus discolor*) learned to discriminate between a virtual echo with constant amplitude and a virtual echo with modulated amplitude. We used the behavioral response of the bats to assess the just noticeable modulation depth, i.e. the threshold. For every bat we extracted one threshold per modulation rate from the psychometric function to form a modulation transfer function across the six modulation rates. It describes the sensitivity of the FM bat *Phyllostomus discolor* for the modulation of echo amplitude.

In the discrimination of constant vs. modulated target, the results of all four bats confirmed our expectations for a psychometric function: The discrimination performance of all bats was best at the largest modulation depth of 0 dB. All bats discriminated the constant target from the amplitude-modulated target at 80%–100% correct choices level. With decreasing modulation depth, the performance deteriorated and eventually dropped to chance level. We observed this relationship for all modulation rates (Fig. 2a–f).

However, the extent of the psychometric function systematically widened towards smaller modulation depths with increasing modulation rate. Starting from the lowest modulation rate of 5 Hz (Fig. 2a), where the average discrimination threshold lay between – 10 and – 12 dB amplitude modulation, the bats' average performance improved with increasing modulation rate. For example, at a modulation rate of 50 Hz the average discrimination threshold lay between – 16 and – 18 dB amplitude modulation (Fig. 2d). At the highest tested modulation rate of 500 Hz, the average discrimination threshold lay between – 18 and – 20 dB amplitude modulation (Fig. 2f).

The just-noticeable modulation values extracted from the six psychometric functions form the modulation transfer function that describes the bats' sensitivity for

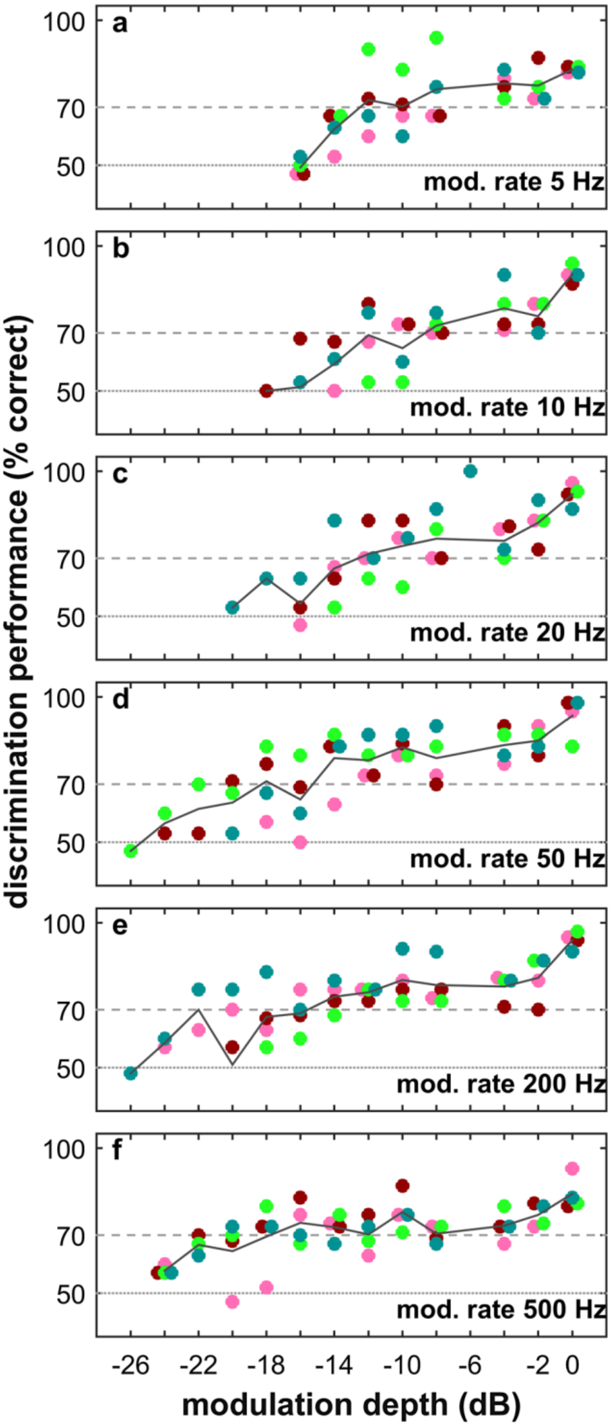

Fig. 2 Psychometric functions of echo-amplitude-modulation discrimination performance at six modulation rates. Each colored dot marks one bat's discrimination performance across 30 trials. Black line plots depict the discrimination performance averaged across bats. Horizontal dashed lines at 50 and 70% correct depict chance and significance level, respectively

echo-amplitude modulation across six modulation rates (Fig. 3). The modulation transfer function shows that overall sensitivity increases with increasing modulation rate: bats performed best at the highest modulation rate of 500 Hz with thresholds around − 20 dB modulation depth. For the lowest modulation rate of 5 Hz, the bats needed around − 10 dB modulation depth to discriminate the constant target from the modulated one.

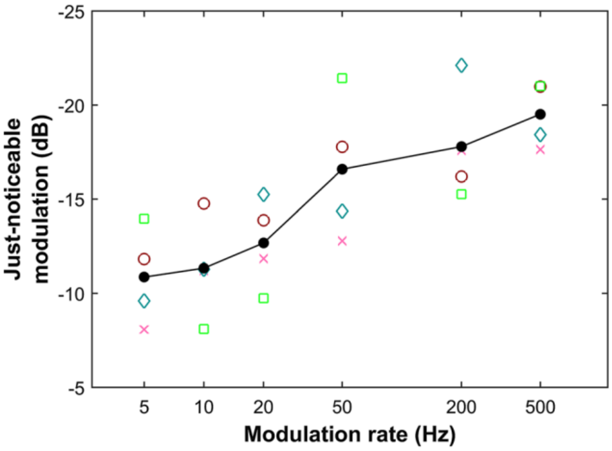

Fig. 3 Echo-amplitude modulation sensitivity. Detection thresholds are generally worst at the lowest modulation rate and improve with increasing modulation rate. Marker colors represent individual bats' thresholds as extracted from sigmoidal fits to the psychometric functions in Fig. 2, the black line connects the mean values of all four bats

Acoustic analyses

The bats' auditory percept depended not only on the echo-acoustic features of the virtual targets themselves but critically on how the bats ensonified them. We performed acoustic analyses of the echolocation calls used by the bats during the behavioral experiment to better understand which sensory- and vocal-motor strategies the bats employed to solve the task. Additionally, we simulated echo properties with respect to the nominal perceptive cue (echo-amplitude modulation) and to possible other perceptive cues.

In the acoustic analysis of the echolocation calls we first determined whether fundamental call parameters like inter-call intervals (ICIs), call duration, or the spectral centroid of the calls changed systematically when the task became more difficult for the bats, i.e., when the modulation depth decreased. We found that the bats did not systematically modify any of these call parameters with increasing task difficulty (Fig. 4a). Second we tested whether these call parameters changed systematically with modulation rate. Here we used only data from those trials where modulation depth was close to the perceptual threshold for this modulation rate and bat. Again, temporal ensonification parameters of the bats remained rather constant as a function of modulation

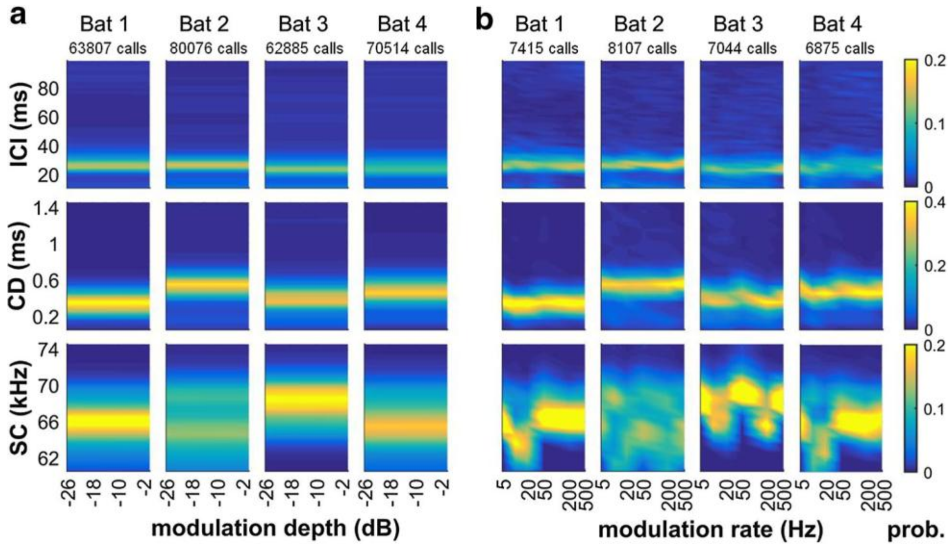

Fig. 4 Temporal and spectral properties of echolocation calls used by the bats for detecting echo amplitude modulations. **a** Effect of modulation depth (= task difficulty). The distribution of inter-call intervals (Row 1), call durations (Row 2) and spectral centroids (Row 3) did not change as a function of modulation depth in any of the four bats (columns). **b** Effect of modulation rate at threshold modulation depth. Data show the extent to which the presented modulation rate lead the animals to adjust ensonification parameters. Again, the distribution of inter-call intervals (Row 1), call durations (Row 2) and spectral centroids (Row 3) did not change systematically as a function of presented modulation rate in the four bats. Residual changes in spectral-centroid distributions result from a change of recording device during data acquisition and do not reflect a change in the bats' ensonification strategy. All data are shown as normalized bin counts with color-coded probability

rate, albeit different across individual bats (Fig. 4b, Rows 1 and 2). The changes in spectral centroid across modulation rates (Fig. 4b, Row 3) do not reflect a change in the bats' ensonification strategy but a change in our recording setup that became necessary during ongoing data acquisition (see "Materials and methods" section). Affected were trials testing modulation rates of 200 Hz, 20 Hz and 10 Hz, depending on the individual bat's progress at the point of the hardware change.

For the simulation of echo properties, we used a sequence of artificial echolocation calls and analyzed amplitude differences between consecutive echoes within the resulting sequence of artificial echoes. The bats' sensitivity for echo-amplitude modulation increased with increasing modulation rate. Therefore the modulation of echo amplitude at threshold decreases with increasing modulation frequency: for modulation rates up to 20 Hz, the maximum amplitude differences between consecutive echoes were around ± 5 dB. For modulation rates of 50 Hz and above, the maximum amplitude differences at threshold decreased to ± 3 dB at 500 Hz (data not shown). *P. discolor* can discriminate echoes from two separate targets that differ in amplitude by 5–7 dB (Heinrich et al. 2011). When at high modulation rates the amplitude differences become smaller, other echo parameters besides the variation of echo amplitude may facilitate the psychophysical task: specifically, bats can analyze variations in the spectral composition of echoes (Schmidt 1988; Weissenbacher and Wiegrebe 2003; Falk et al. 2011). In another simulation, we tested whether fast amplitude modulations can induce perceivable changes in echo spectral composition. We analyzed the frequency content of the echoes and found that depending on the phase of the modulation relative to the timing of the call emission, the target amplitude modulation can indeed affect the echo spectrum (Fig. 5). The reason is that the time-variant amplitude of the virtual target interacts with the time-variant frequency content of the echolocation call: when at echo onset the virtual-target amplitude is high, the high frequencies of the echoes are stressed. When, however, at echo onset the target amplitude is low and increasing during the echo, the low frequencies of the echo are stressed. These target-amplitude induced variations in echo spectral content can only come about when the modulation period (1/modulation rate) is in the range of the echo duration. Moreover, the changes in echo spectrum depended on the phase of the modulation, so that consecutive echoes were subjected to different frequency changes.

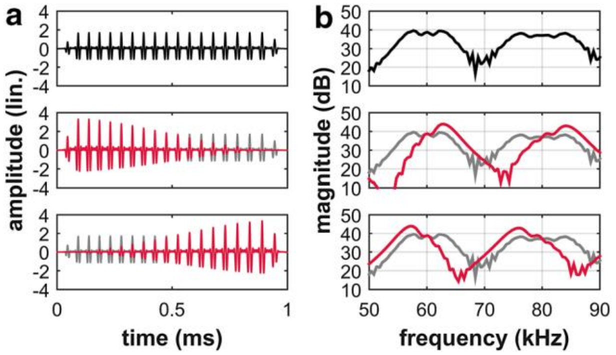

Fig. 5 Simulated time signals (**a**) and zoomed-in power spectra (**b**) of echoes from an unmodulated reflector (top row), from a reflector with falling amplitude (middle row) and from reflector with rising amplitude (bottom row). **a** When the modulation rate is high enough, i.e. the modulation period is about the length of the echo, the echo amplitude changes significantly during the duration of one echo. **b** Due to the downward frequency modulation of the call, i.e. high frequencies are followed by intermediate and then low frequencies within the call's duration, different frequency bands get emphasized and suppressed in modulated echoes (red) in comparison to the unmodulated echo (black/gray)

Discussion

When a bat echolocates towards a real target, the delay and amplitude of the echo change together, dependent on the distance of the target. Here we show that FM bats are very sensitive to modulations in amplitude of echoes: *Phyllostomus discolor* bats were well able to distinguish a virtual target with constant target strength from a virtual target whose target strength was modulated over time. To the best of our knowledge, this study presents the first evidence of an echolocating FM bat detecting a target movement based solely on the modulation of echo-amplitude.

Amplitude-modulation sensitivity

The capability of FM bats to perceive amplitude modulations in echo sequences does not come as a complete surprise (Griffin 1958; Roeder 1963). But our results are the first to experimentally support the implications of theoretical studies on perception of motion with FM echolocation. In those studies, fluttering insects were ensonified with a sequence of synthetic FM signals, and information on wing beat cycle could be decoded from the resulting sequence of echoes (Kober and Schnitzler 1990; Moss and Zagaeski 1994).

The neural adaptations to process echo-amplitude information have been established for the FM bat *Myotis lucifugus* (Condon et al. 1994): neurons in the inferior colliculus faithfully represent amplitude modulations imposed on a sequence of artificial echolocation calls up to modulation rates around 100 Hz. However, there are three fundamental differences between the electrophysiological experiment by Condon et al. (1994) and the current psychophysical experiment. First, we varied modulation depth to determine a perceptual threshold while Condon et al. always applied 100%

(0 dB) modulation depth. Second, the carrier was always a periodic pulse train, whereas in our experiments, the bats themselves determined the ensonification pattern, producing pulses in strobe groups rather than periodically. Third, the combination of pulse rate and modulation rate was always chosen to deliberately avoid a wagon wheel effect in the physiological studies. In summary, this electrophysiological study investigated the effect of amplitude modulation on the neural representation of echo sequences, but the limitations of the electrophysiological protocol preclude a direct comparison with the current psychophysical data.

P. discolor bats were much more sensitive to higher modulation rates than to lower modulation rates: for the lowest modulation rate of 5 Hz, the bats needed around − 10 dB modulation depth to discriminate the constant target from the modulated one, whereas they still detected modulations around − 20 dB modulation depth at the highest modulation rate of 500 Hz (Fig. 3). Strikingly, our findings that sensitivity increased with increasing modulation rate are inconsistent with two related findings: First, they differ from what we found about sensitivity to modulation of echo delay (Baier and Wiegrebe this issue). This suggests that time-variant information on delay and on amplitude of echoes is processed differently. Second, the results are the opposite of what has been found in studies where subjects passively listened to amplitude-modulated sounds (Fay and Wilber 1989). With broadband carrier stimuli, modulation transfer functions in these experiments are always low-pass, i.e., subjects are less sensitive to high modulation rates than to low modulation rates. This is in direct contrast to the results of the current, active echolocation study. We will discuss both points in detail after addressing the bats' acoustic signals.

Acoustic analyses

As expected from the delay study (Baier and Wiegrebe this issue), individual bats maintained the same call parameters throughout the experiment (Fig. 4). In fact, call parameters were identical to the ones used by the bats in the delay study: we found call durations around 0.4 ms and inter-call intervals (ICIs) around 29 ms. We propose that call parameters were tied to the here simulated target distance of 72 cm, although the bats were not in a formal target-approach situation, where a systematic decrease in ICI and call duration occurs (Griffin et al. 1960). We can ask whether changing the bat's perceived target distance will result in different call parameters and lead to a different performance in the detection task. Echo sound pressure level (SPL) influences the performance in a range detection task (Denzinger and Schnitzler 1994, 1998). The sensitivity of neurons to sinusoidal amplitude modulation (SAM) is linked to the sound level of the carrier tone (Schuller 1979; Ostwald et al. 1988). Since bats could move freely in our experimental setup, we did not attempt to measure echo SPL. However, we had designed our virtual targets as plain reflectors that very likely yielded target strengths large enough to not affect the modulation detection. The question whether our chosen target range did so remains open.

Comparison to echo-delay modulation

In the companion paper, we have investigated sensitivity for the modulation of echo delay (Baier and Wiegrebe this issue). We found that *P. discolor* bats were very sensitive to low and high delay-modulation rates and much less so to intermediate delay-modulation rates around 20 and 50 Hz. We proposed that for delay-modulation rates of 20 Hz and higher, bats suffer from an auditory wagon-wheel effect because their call repetition rate matches the modulation rate of the target or an integer multiple thereof. In these cases, the difference in echo delay between consecutive echoes becomes undetectably small. The use of spectral cues that only occur at high modulation rates and are not affected by this wagon-wheel effect can explain the recovery of detection performance for modulation rates of 100 Hz and higher. If we assume that for the detection of echo-amplitude modulation bats use amplitude differences between consecutive echoes, we should observe a similar wagon-wheel effect for the current sensitivity for amplitude modulation. However, we did not observe a drop in performance at intermediate amplitude-modulation rates (Fig. 3). This indicates that the currently observed increasing sensitivity for increasing amplitude-modulation rates does not purely reflect the ability to detect echo-amplitude differences. It rather indicates that the bats employ a different detection strategy already at low modulation rates where the wagon-wheel effect does not yet occur.

We propose a detection strategy that depends on spectral cues rather than amplitude cues. We simulated the interaction of a frequency-modulated echolocation call with a fast amplitude modulation and observed that in an amplitude-modulated echo, different frequency bands get emphasized and suppressed in comparison to an unmodulated echo (Fig. 5). We quantified these changes by calculating the weighted mean of the frequencies present in the echo, i.e., the echo spectral centroid. The variation in echo amplitude between different phases of the amplitude modulation decreased with increasing modulation rates (Fig. 6, black line). In direct contrast to this, variation in spectral centroid between different phases of the amplitude modulation increased with increasing modulation rates (Fig. 6, gray line). In other words, amplitude cues become less and spectral cues become more available with increasing modulation rate. The bats' behavioral performance in detecting amplitude modulations can therefore be explained by a shift in the emphasis that they place on processing different auditory

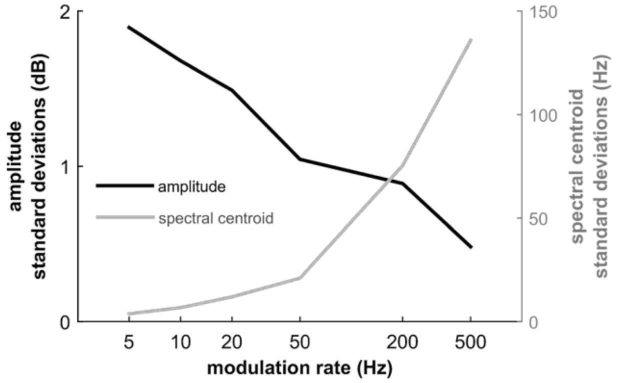

Fig. 6 Standard deviations for amplitude and for spectral centroid of echoes from an artificial echolocation call at eight different modulator phases. The amount of variation in the amplitude of modulated echoes decreases with modulation rate (black line). In contrast, the amount of variation in the spectral centroid of modulated echoes increases with modulation rate (gray line). The modulation depth of the modulator for each modulation rate was set to the respective average detection threshold (black markers in Fig. 3)

cues. We can only guess at the details of the perceptual weighting that the bats might apply towards amplitude and spectral (and possibly further) cues, however, the high-pass shape of the behavioral modulation transfer function suggests a strong contribution of the spectral cues.

The idea that the perception of echo-amplitude modulation is processed differently from the perception of echo-delay modulation is corroborated by observations during the training procedure: all four bats that participated in the current amplitude-modulation experiments had previously participated in the delay-modulation experiments. The rewarded stimulus in these two experiments was the same unmodulated reflection. The bats were thus familiar with virtual targets, the experimental set-up and procedure. However, while these bats needed very little retraining when we wanted to acquire data for a new delay modulation rate, the same individuals needed extensive retraining periods when we wanted to acquire data for a new amplitude modulation rate. Retraining periods are illustrated in Fig. 7. The data clearly show that on average the animals needed more than five times the retraining time when we changed the amplitude-modulation rate than when we changed the delay-modulation rate. These data, together with the difference in psychophysical performances suggest that the modulation of echo amplitude may be processed fundamentally differently from the modulation of echo delay. We hypothesize that the dedicated neural circuitry for echo delay, and specifically the topographic representation of echo delay in the bat auditory cortex, supports delay-modulation detection (Hagemann et al. 2010; O'Neill and Suga 1979; Dear et al. 1993), whereas the lack of a similar topographic representation for echo amplitude may underlie the much more demanding training and data acquisition for amplitude-modulation detection. Modulation at high rates induces spectral cues for both echo-delay modulation and echo-amplitude modulation. These spectral cues are readily represented along the tonotopic axes at virtually all stages of the bat auditory system. The fact that our bats learned to detect fast delay modulations much quicker than fast amplitude modulations may be related to the fact that the delay-induced Doppler distortions create overall much more dramatic spectral distortions than the above described spectral changes induced by the echo-amplitude modulation.

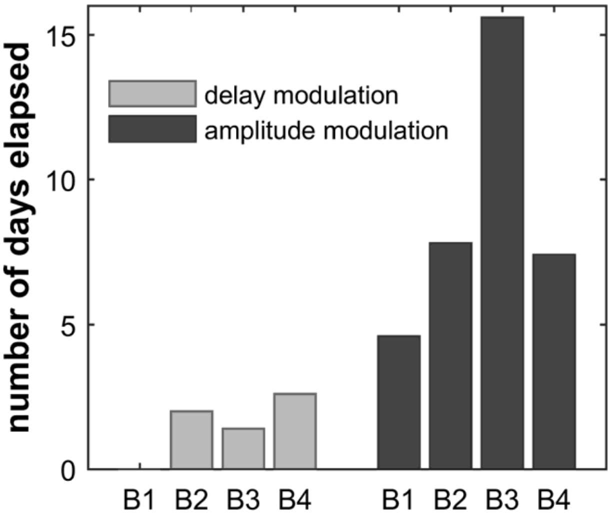

Fig. 7 Average time between start of training and start of data collection for delay-modulation and amplitude-modulation experiments. During the echo-delay modulation experiment, it took the bats on average 1.5 days to learn a new modulation rate (light bars, $n = 4$). During the echo-amplitude modulation experiment, the same individual bats needed on average 8.9 days to learn a new modulation rate (dark bars, $n = 4$). B1–B4 refers to bats, the same individuals in both experiments

Comparison to passive listening

In passive listening, studies that investigate sensitivity to amplitude modulation typically use broadband noise as the carrier signal for the modulation, as opposed to a sequence of echoes of self-produced, broadband echolocation calls. The sensitivity of mammals and birds to amplitude modulation of noise consistently becomes worse with increasing modulation rate (Viemeister 1979; Salvi et al. 1982; Burdin et al. 1973; Dooling and Searcy 1981).

These findings are in direct contrast to the results we present here for the sensitivity of bats to amplitude modulation of echoes, and indicate separate processing mechanisms for amplitude modulations in passive and active listening, at least for part of the range of modulation rates. This discrepancy can be assigned to the fundamental difference in the nature of the modulated sounds. In passive listening, the carrier of the amplitude modulation is a continuous signal that persistently reflects every point of the modulation. In active listening, the carrier of the amplitude modulation is a transient, i.e. impermanent signal that reflects only momentary stages of the modulation.

When the carrier of the amplitude modulation in passive listening is a pure tone, there also arise spectral cues that allow the detection sensitivity of human listeners to recover for modulation rates higher than 50 Hz (Viemeister 1979). Fast amplitude modulation of a narrowband signal creates spectrally resolvable sidebands: when a 1000 Hz pure tone is amplitude-modulated at a modulation rate of 200 Hz, the sidebands are at 800 and 1200 Hz. If we regarded the frequency-modulated broadband echolocation call as an assembly of pure-tone carriers, the broadband echo itself would mask any emerging frequency sidebands, even at the highest presented amplitude modulation rate of 500 Hz. Instead, the observed changes in the frequency spectrum of amplitude-modulated echoes (Figs. 5, 6) are likely to serve the same purpose as frequency sidebands in passive listening, complementing the detection of echo-amplitude modulation for those (high) modulation rates where this strategy is more efficient than resolving temporal amplitude differences.

Dankiewicz et al. (2002) performed the only other perceptual study on amplitude modulation detection with echolocation, i.e. not in passive listening. They trained a bottlenose dolphin (*Tursiops truncatus*) to discriminate modulated synthetic echoes from unmodulated ones. In contrast to the current work, the authors did not generate real-time echoes from the animal's emissions but only used the animal's emission to trigger the playback of the synthesized echo. Second, the authors applied one amplitude modulation cycle across a fixed number of echoes (8 to 64), not across a fixed time; thus the effective modulation frequency depended on the number of the dolphin's emissions per time. Modulation detection thresholds changed from about 1.2% (= – 38 dB) for an effective modulation frequency around 2 Hz to a threshold of about 6% (= – 24 dB) for an effective modulation frequency around 16 Hz. Thus the modulation transfer function was low-pass, similar to what has been reported for amplitude-modulation detection with wideband noise carriers in humans (Viemeister 1979). Although the authors did not test the dolphin at effective modulation rates higher than 16 Hz, the general potential for spectral echo changes is low: interactions between the amplitude modulation of the virtual target and the frequency modulation of the emitted call are limited in the dolphin, because its average echo duration is markedly shorter than in our bats [128 µs and 500 µs, respectively (Dankiewicz et al. 2002)]. Instead, with regard to perceiving amplitude differences between consecutive echoes at very small modulation depths, echolocating dolphins are presumably superior to bats, as they have repeatedly been reported to be capable of detecting a 1 dB difference in target strength (Evans 1973; Bullock et al. 1968; Johnson 1967; Moore et al. 1995) compared to 5–7 dB in *P. discolor* (Heinrich et al. 2011). Additionally, dolphins may have no need to detect modulation rates that would be high enough to create spectral cues. While echo-amplitude modulation can reflect the changing orientation of the target relative to the emitter both in bats and dolphins, the speed of these orientation changes is likely much slower underwater than in air. All of these aspects make dolphins more likely to solely use echo-amplitude cues, resulting in their reported low-pass modulation transfer function.

As opposed to dolphins, FM bats would greatly benefit from a mechanism to facilitate flutter detection at high modulation rates, because these make up a large portion of insect prey wing beat rates (Pringle 1949; Sotavalta 1953; Gibson et al. 2010). Bats from the Eocene radiation were nocturnal and insectivore (Speakman 2001; Veselka et al. 2010). The surprisingly good performance of our bats (especially at higher modulation rates) leads us to hypothesize that the biophysical properties of FM echolocation calls do indeed facilitate insect flutter detection, not only based on echo-delay modulations, but also based on echo-amplitude modulations. We suggest that the frequency-modulated call structure does not only reflect a motor constraint of laryngeal echolocation or a means to minimize Doppler-distortion-induced misjudgments in echo delay (Altes 1995; Simmons et al. 2004). We argue that adaptive selection to insect prey motion shaped FM echolocation calls not into a perfect Dirac Impulse but into a structure that converts fast amplitude modulations into spectral cues, which are readily represented along the bats' tonotopic axis. We surmise that the small deviation from the Dirac structure grants FM bats sensitivity to fast-changing time-variant environments together with high spatial acuity in time-invariant scenarios.

In summary, our work offers insights into the processing of modulated echo amplitude by FM bats. We have introduced a virtual-reality approach with time-variant targets to assess sensitivity to echo-amplitude modulation independently of echo-delay modulation. We have shown that FM bats are well capable of detecting modulations of echo amplitude despite the limitations that arise from the use of very short echolocation signals. We suggest that amplitude-modulation detection with echolocation not only differs fundamentally from delay-modulation detection, but also from amplitude-modulation detection in passive listening, due to the transient nature of the carrier signal. We speculate that the mechanism to detect (particularly the fast) modulations does not rely on the nominal cue alone, the echo amplitude, but on spectral cues that occur when the frequency modulation of the echolocation call interacts with the amplitude modulation of the target. Although we do not yet know whether FM bats make use of echo-amplitude modulations when they encounter a moving target, we provide an important proof-of-principle demonstration that offers release from the supposed trade-off between temporal and spatial acuity for FM bats.

Acknowledgements We thank H. Goerlitz, H. Peremans, F. Rosales, and the Wiegrebe lab for discussion and D. Dechmann and K. Safi for valuable comments on earlier versions of the manuscript. We thank two anonymous referees whose helpful comments greatly improved this manuscript. We are grateful to E. Lattenkamp and M. Linnenschmidt for help during data acquisition. We thank B. Grothe for providing excellent research infrastructure. All experiments were conducted under the regulations of the German Law on Animal Protection (approval 55.2-1-54-2532-34-2015, Regierung von Oberbayern).

Author contributions ALB and LW conceived and designed the study. KS performed the experiment. ALB and LW analyzed the data. ALB wrote the manuscript with assistance from LW. All authors discussed the analysis and interpretation of the results.

Funding This work was supported by the Deutsche Forschungsgemeinschaft (DFG WI 1518/13 – 1 to LW). ALB was supported by the International Max Planck Research School for Organismal Biology.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Altes RA (1995) Signal processing for target recognition in biosonar. Neural Netw 8(7–8):1275–1295

Bullock TH, Grinnell AD, Ikezono E, Kameda K, Katsuki Y, Nomoto M, Sato O, Suga N, Yanagisawa K (1968) Electrophysiological studies of central auditory mechanisms in cetaceans. Z Vergl Physiol 59(2):117–156. https://doi.org/10.1007/bf00339347

Burdin V, Markov V, Reznik A, Skornyakov V, Chupakov A (1973) Determination of the just noticeable intensity difference for white noise in the Black Sea bottlenose dolphin (*Tursiops truncatus ponticus* Barabasch). Morphol Ecol Mar Mamm:169–173

Condon CJ, White KR, Feng AS (1994) Processing of amplitude-modulated signals that mimic echoes from fluttering targets in the inferior colliculus of the little brown bat, *Myotis lucifugus*. J Neurophysiol 71(2):768–784

Dankiewicz LA, Helweg DA, Moore PW, Zafran JM (2002) Discrimination of amplitude-modulated synthetic echo trains by an echolocating bottlenose dolphin. J Acoust Soc Am 112(4):1702–1708. https://doi.org/10.1121/1.1504856

Dear SP, Fritz J, Haresign T, Ferragamo M, Simmons JA (1993) Tonotopic and functional organization in the auditory cortex of the big brown bat, *Eptesicus fuscus*. J Neurophysiol 70(5):1988–2009. https://doi.org/10.1152/jn.1993.70.5.1988

Denzinger A, Schnitzler H-U (1994) Echo SPL influences the ranging performance of the big brown bat, *Eptesicus fuscus*. J Comp Physiol A 175(5):563–571. https://doi.org/10.1007/bf00199477

Denzinger A, Schnitzler H-U (1998) Echo SPL, training experience, and experimental procedure influence the ranging performance in the big brown bat, *Eptesicus fuscus*. J Comp Physiol A 183(2):213–224. https://doi.org/10.1007/s003590050249

Dooling RJ, Searcy MH (1981) Amplitude modulation thresholds for the parakeet (*Melopsittacus undulatus*). J Comp Physiol A 143(3):383–388

Evans WE (1973) Echolocation by marine delphinids and one species of freshwater dolphin. J Acoust Soc Am 54:191–199

Falk B, Williams T, Aytekin M, Moss CF (2011) Adaptive behavior for texture discrimination by the free-flying big brown bat, *Eptesicus fuscus*. J Comp Physiol A 197(5):491–503. https://doi.org/10.1007/s00359-010-0621-6

Fay RR, Wilber LA (1989) Hearing in vertebrates: a psychophysics databook, vol 86. Hill-Fay Associates, Winnetka. https://doi.org/10.1121/1.398550

Fenton MB, Faure PA, Ratcliffe JM (2012) Evolution of high duty cycle echolocation in bats. J Exp Biol 215(Pt 17):2935–2944. https://doi.org/10.1242/jeb.073171

Gibson G, Warren B, Russell IJ (2010) Humming in tune: sex and species recognition by mosquitoes on the wing. JARO 11(4):527–540. https://doi.org/10.1007/s10162-010-0243-2

Greiter W, Firzlaff U (2017) Echo-acoustic flow shapes object representation in spatially complex acoustic scenes. J Neurophysiol 117(6):2113–2124

Griffin DR, Webster FA, Michael CR (1960) The echolocation of flying insects by bats. Anim Behav 8(3):141–154. https://doi.org/10.1016/0003-3472(60)90022-1

Hagemann C, Esser K-H, Kössl M (2010) Chronotopically organized target-distance map in the auditory cortex of the short-tailed fruit bat. J Neurophysiol 103(1):322–333. https://doi.org/10.1152/jn.00595.2009

Hechavarría JC, Macías S, Vater M, Voss C, Mora EC, Kössl M (2013) Blurry topography for precise target-distance computations in the auditory cortex of echolocating bats. Nat Commun 4:2587

Heinrich M, Warmbold A, Hoffmann S, Firzlaff U, Wiegrebe L (2011) The sonar aperture and its neural representation in bats. J Neurosci 31(43):15618–15627. https://doi.org/10.1523/jneurosci.2600-11.2011

Johnson CS (1967) Sound detection thresholds in marine mammals. Mar Bioacoust 2:247–260

Kober R, Schnitzler HU (1990) Information in sonar echoes of fluttering insects available for echolocating bats. J Acoust Soc Am 87(2):882–896. https://doi.org/10.1121/1.398898

Measor K, Yarrow S, Razak KA (2018) Topography of sound level representation in the FM sweep selective region of the pallid bat auditory cortex. Hearing Res 367:137–148. https://doi.org/10.1016/j.heares.2018.05.017

Moore PW, Pawloski DA, Dankiewicz L (1995) Interaural time and intensity difference thresholds in the bottlenose dolphin (*Tursiops truncatus*). Sens Syst Aqu Mamm:11–23

Moss CF, Surlykke A (2010) Probing the natural scene by echolocation in bats. Front Behav Neurosci 4:33. https://doi.org/10.3389/fnbeh.2010.00033

Moss CF, Zagaeski M (1994) Acoustic information available to bats using frequency-modulated sounds for the perception of insect prey. J Acoust Soc Am 95(5):2745–2756. https://doi.org/10.1121/1.409843

Neuweiler G (1984) Foraging, echolocation and audition in bats. Naturwissenschaften 71(9):446–455

Nowak RM (1994) Walker's bats of the world. JHU Press, Baltimore

O'Neill W, Suga N (1979) Target range-sensitive neurons in the auditory cortex of the mustache bat. Science 203(4375):69–73. https://doi.org/10.1126/science.758681

Ostwald J, Schnitzler H-U, Schuller G (1988) Target discrimination and target classification in echolocating bats. In: Nachtigall PE, Moore PWB (eds) Animal sonar: processes and performance, Springer, Boston, pp 413–434

Pringle J (1949) The excitation and contraction of the flight muscles of insects. J Physiol 108(2):226–232

Salvi RJ, Giraudi DM, Henderson D, Hamernik RP (1982) Detection of sinusoidally amplitude modulated noise by the chinchilla. J Acoust Soc Am 71(2):424–429. https://doi.org/10.1121/1.387445

Schmidt S (1988) Discrimination of target surface structure in the echolocating bat, *Megaderma lyra*. In: Nachtigall PE, Moore PWB (eds) Animal sonar: processes and performance, Springer, Boston, pp 507–511. https://doi.org/10.1007/978-1-4684-7493-0_52

Schnitzler H-U, Hackbarth H, Heilmann U, Herbert H (1985) Echolocation behavior of rufous horseshoe bats hunting for insects in the flycatcher-style. J Comp Physiol A 157(1):39–46. https://doi.org/10.1007/bf00611093

Schuller G (1979) Coding of small sinusoidal frequency and amplitude modulations in the inferior colliculus of 'CF-FM' bat, *Rhinolophus ferrumequinum*. Exp Brain Res 34(1):117–132

Simmons JA, Neretti N, Intrator N, Altes RA, Ferragamo MJ, Sanderson MI (2004) Delay accuracy in bat sonar is related to the reciprocal of normalized echo bandwidth, or Q. Proc Natl Acad Sci USA 101(10):3638–3643

Sotavalta O (1953) Recordings of high wing-stroke and thoracic vibration frequency in some midges. Biol Bull 104(3):439–444

Speakman JR (2001) The evolution of flight and echolocation in bats: another leap in the dark. Mamm Rev 31(2):111–130

Veselka N, McErlain DD, Holdsworth DW, Eger JL, Chhem RK, Mason MJ, Brain KL, Faure PA, Fenton MB (2010) A bony connection signals laryngeal echolocation in bats. Nature 463(7283):939

Viemeister NF (1979) Temporal modulation transfer functions based upon modulation thresholds. J Acoust Soc Am 66(5):1364–1380. https://doi.org/10.1121/1.383531

Weissenbacher P, Wiegrebe L (2003) Classification of virtual objects in the echolocating bat, *Megaderma lyra*. Behav Neurosci 117(4):833–839. https://doi.org/10.1037/0735-7044.117.4.833

DISCUSSION

In our opening example, we observed bats foraging low over a lake. All water surfaces are characterised by their co-varying spatial and temporal frequencies. This thesis aimed to quantify the sensitivity of bat echolocation for spatial and temporal frequency. I assessed sensitivity for depth contrast as a function of spatial frequency in Chapter 1. I measured sensitivity for echo-delay modulations and for echo-amplitude modulations as a function of temporal frequency in Chapters 2 and 3, respectively. Chapter 1 demonstrated a high-pass filter for the detection of spatial frequency. Chapter 2 revealed an echo-acoustic wagon-wheel effect that arises from the interaction of call rate and modulation rate. Furthermore, Chapter 2 introduced the possible use of Doppler distortions in FM echolocation. Chapter 3 corroborated the use of Doppler cues but indicated a fundamental difference in the processing of modulations of echo delay on the one hand and echo amplitude on the other hand. On the whole, these data help to improve our understanding of how bats perceive complex environments with a non-spatial, non-continuous sensory system. Below I discuss these results in a broader context and indicate research directions that arose from the current experimental work. Some of them I have begun to address, but they ultimately proved to be beyond the scope of the current thesis.

Water turbulence: clutter or clue? **-** Water bodies offer an abundance of prey, often soft bodied and easily digestible (Fukui et al. 2006). Many bat species exploit this rich resource and show morphological adaptations to capture prey from water surfaces or even out of the water: their hind legs and tail membranes are highly specialized and fish-eating species have sharp claws. We find these so-called trawling bats in at least three bat families: Vespertilionidae [*Myotis adversus* (Thompson and Fenton 1982), *Myotis albescens* (Kalko et al. 1996), *Myotis capaccinii* (Kalko 1990), *Myotis dasycneme* (Britton et al. 1997), *Myotis daubentonii* (Kalko and Schnitzler 1989), *Myotis ricketti* (Ma et al. 2003), *Myotis vivesi* (Blood and Clark 1998)], Noctilionidae [*Noctilio albiventris* (Kalko et al. 1998), *Noctilio leporinus* (Schnitzler et al. 1994)] and Phyllostomidae [*Macrophyllum macrophyllum* (Weinbeer et al. 2006)].

Remarkably, these bats don't show adaptations for clutter avoidance (Jones and Rayner 1991; Kalko et al. 1998; Siemers et al. 2001a; Jones and Rydell 2003; Siemers and Schnitzler 2004). In cluttered environments efficient prey detection within the acoustic domain is especially challenging. Clutter refers to structures the echoes of which can mask the prey echo. Bats that forage close to vegetation or other clutter usually apply one of two strategies to increase signal-to-noise ratio. Either they adapt their echolocation call to gain maximal resolution and thereby increase the signal (Schnitzler and Kalko 2001; Siemers and Schnitzler 2004). Or, they use passive listening to prey-generated sounds instead of echolocation (Jones and Rydell 2003) and thereby reduce the noise. As a matter of fact, smooth water surfaces do not require either of these adaptations to clutter as they are considered clutter-free (Kalko and Schnitzler 1989).

Foraging trawling bats fly at low heights above the water surface and emit their calls in forward direction. The sound beam interacts with the bat's immediate environment in three different ways: First, smooth water surfaces act like mirrors, both in vision and echolocation. Sound waves that hit the water surface at small angles relative to the surface are reflected away from the bat, according to Snell's law. Therefore, little or no clutter echo is reflected back to the bat (Mackey and Barclay 1989; Schnitzler et al. 1994; Boonman et al. 1998; Rydell et al. 1999). Second, the small fraction of the sound beam that hits the water perpendicularly, directly below the bat, produces a detectable echo. The delay of this echo encodes the bat's flight height above the water surface. Finally, sound waves that hit a target floating on the water surface in front of the bat are reflected back towards the bat in a twofold way: directly, and indirectly via the water surface. Hence, the overall amplitude and duration of this combined echo is larger than the direct echo alone (as it would be produced by the same target suspended in air). Due to this acoustic mirror effect, bats can detect targets on smooth water surfaces easier (Boonman et al. 1998; Siemers et al. 2001b) and from further away (Siemers et al. 2005) than on vegetation or suspended in air. In a nutshell, calm smooth water surfaces render prey items very conspicuous.

FIGURE D.1 **MYOTIS DAUBENTONII, *A* *EUROPEAN TRAWLING BAT.*** *Photo by D. Nill*

Indeed, foraging trawling bats prefer smooth water to vegetation-covered water (Boonman et al. 1998), water covered by artificial foam blocks (Mackey and Barclay 1989), or turbulent water (Von Frenckell and Barclay 1987; Rydell et al. 1999; Warren et al. 2000). In most cases, this preference cannot be explained by prey abundance (Von Frenckell and Barclay 1987; Boonman et al. 1998; Rydell et al. 1999). However, Warren et al. (2000) demonstrate that insect abundance correlates with the habitat preferences of bats. Generally, it has been assumed that clutter and/or noise produced by the water turbulences impair bats' foraging efficiency.

A more recent study, however, indicates that prey detection is not impaired by turbulent water: Zsebők et al. (2013) formally quantified the effects of surface properties and target height on both target detection and discrimination of *Myotis daubentonii*. Experiments were carried out both in a large flight room under controlled conditions and in the field in a more natural situation. In a two-alternative, forced-choice paradigm, the bats had to detect a mealworm and discriminate it from an inedible dummy. Echolocation and flight behaviour were recorded. Psychophysical performance was measured as a function of height above either a smooth or a cluttered surface. The clutter surface consisted of artificial lawn, which contains strong high spatial-frequency components. At low target heights (<35 cm), the bats' detection performance was worse over clutter than over the smooth surface, whereas at a target height of 50 cm there was no effect of surface structure on target detection. Target discrimination was also impaired at low heights above the cluttered surface. Generally, over clutter, bats produced approach calls with significantly higher peak frequency and flew about 20 cm higher than when approaching the target above water. However, prey detection performance above the rippled water in the field experiments was very similar to that above completely calm water in the lab experiments.

These discrepancies of the studies on trawling above turbulent water suggest that the degree of turbulence (and thus the spatial-frequency composition) determines the degree of prey detection impairment. I speculate that the auditory system of echolocating bats uses the concept of spatial frequency in order to segregate foreground from background and thereby increase signal-to-noise ratio during prey detection over turbulent water surfaces. I propose the existence of a threshold spatial frequency below which turbulent water surfaces don't impair prey detection and above which turbulent water surfaces impair prey detection with echolocation and are therefore avoided during foraging.

The artificial grass used by Zsebők et al. (2013) consisted mainly of sharp edges and thus reduced detection performance. The clutter surface used by Siemers and Schnitzler (2004) and the duckweed vegetation of Boonman et al. (1998) included regular sharp edges as well. Thus, sharp edges (i.e. high spatial frequencies) in the background structure dramatically reduce performance of bats. In contrast, *M. daubentonii* perform similarly above smooth water in the laboratory and above rippled water in the field. The echoacoustic reflection of rippled water did not impair the bats' performance (Zsebők et al. 2013). According to my results in Chapter 1, water surfaces with low spatial frequency waves can be perceived as smooth surfaces and hence do not affect foraging performance. Rydell et al. (1999) report reduced activity of *M. daubentonii* over water with ripples that were approximately 2-3 cm in amplitude and 5-10 cm in wavelength. This corresponds to spatial frequencies of 10-20 cyc/m and grating depths of ±10 to ±15mm. In Chapter 1, bats showed a detection threshold of around ±4mm for a spatial frequency of 17.8 cyc/m (0.5 cyc/deg), meaning that the ripples that Rydell and his colleagues observed would have been well perceivable and indeed have affected prey detection. On large water bodies, wind generates waves that typically have similarly high amplitudes (0.8-38.3 mm) but much longer wave lengths of around 82 cm, corresponding to a spatial frequency of less than 1.5 cyc/m [wave maximum frequencies around 1.4 Hz with an upper-frequency limit below 10 Hz (Bleckmann and Rovner 1984)]. Based on the results from Chapter 1, bats are highly unlikely to perceive such low spatial frequencies, no matter the wave amplitude. However, the exact cut-off frequency of the spatial high-pass filter is very likely to be species-dependent.

So far I have discussed water turbulences as possible hindrance for foraging bats. But many predatory species across the animal kingdom make use of water surface ripples generated by their prey.

A positive tactic response towards surface waves has been demonstrated in leeches (Dickinson and Lent 1984), surface-feeding fish (Bleckmann et al. 1989), a variety of aquatic amphibians and insects (Bleckmann 1985), and fishing spiders (Bleckmann et al. 1994). Most of these predators can discriminate between the different wave-sources by means of analysing both spatial and temporal frequency and amplitudes of the waves. Objects falling onto the water surface elicit transient changes in both spatial and temporal frequency. Drowning terrestrial insects produce wave stimuli that are characterized by broad frequency bands (half-amplitude bandwidth 30-60 Hz), high upper-frequency limits (100-140 Hz), and low amplitudes (peak amplitudes 2-81 µm) with the peak amplitude situated far above 10 Hz (Lang 1980; Bleckmann 1985). Some specialist species can also peruse the wavefront curvature, the surface's dispersive properties and the frequency-dependant absorption (Bleckmann and Schwartz 1982; Hoin-Radkovsky et al. 1984; Bleckmann et al. 1994). While insect-generated waves are minuscule in amplitude, their spatial frequency is large, far beyond the range we could test in the Chapter 1 experiment. It would be interesting to know if *P. discolor* and other FM bats can perceive ripples of these qualities.

There are two known examples of exploiting prey-generated turbulence for prey detection among bats: *Noctilio leporinus*, which uses CF echolocation calls (Schnitzler et al. 1994; Ubernickel et al. 2013), and the neotropical fringe-lipped bat *Trachops cirrhosus* (Halfwerk et al. 2014), a Phyllostomid bat that uses FM echolocation. *T. cirrhosus* forages mainly on male túngara frogs (*Physalaemus pustulosus*), hence it is also called the frog-eating bat. Male túngara frogs advertise for females from small breeding puddles. The main component of the mating signal is acoustic. The frog produces either a simple call, consisting of a frequency-modulated ~350 ms sweep called a 'whine', or a complex call consisting of a whine followed by 1-7 broadband ~40–80 ms harmonic bursts called 'chucks' (Ryan 1980).

Female frogs use the call to locate a mate and frog-eating bats rely on the call to locate a meal. Both receivers are preferentially attracted to complex calls over simple calls. In the frog's display there are also associated visual cues (an inflating vocal sac, perceived by the bats by echolocation), and turbulence cues (water ripples generated by the inflating vocal sac). To reduce predation risk, frogs stop calling in response to predator cues (Bernal et al. 2007), a strategy that effectively increases localization errors by bats (Page and Ryan 2008). However, ripple propagation continues for 2-3 s after call cessation (Halfwerk et al. 2014) and thus could be used as additional foraging cue. Even though *T. cirrhosus* mainly listens to prey-generated cues for detection and localization, it produces echolocation calls throughout the prey approach (Barclay et al. 1981). *T. cirrhosus* increases its attack rate on attack model frogs by 36.5 % when ripples are added to the acoustic component of the call (Halfwerk et al. 2014).

I was interested in the attack behaviour of the frog-eating bat when presented with artificial water waves mimicking the ripples created by the inflating vocal sac of the túngara frog. In order to learn more about how *Trachops cirrhosus* might exploit the turbulences generated by the frog, we designed a set of experiments to quantify attack rates on water ripples in the absence of additional prey cues (inflating vocal sac or frog call). Single wild bats were captured and left to forage above an artificial pond in an outdoor flight cage. Water ripples were generated in one of four possible locations on the pond and bats received a food reward in case of successful detection. Echolocation behaviour was recorded with a five-channel microphone array. Because bats do not initiate foraging flights without the broadcast of a frog call (Page and Ryan 2008), a speaker was placed next to the pond that broadcasted a call at the beginning of a trial and was turned off as soon as the bat left its perch. Out of twelve captured bats four learned to search for the food reward on the pond. None of these four bats detected the artificial turbulences at a significant success rate although two bats showed a positive trend towards finding the correct location. Thus, the question whether the frog-eating bat is able to find prey based solely on water turbulence cues remains open. Based on my observations during the experiments, I suspect a strong influence of the acoustic cue on the behaviour of the bat. I hypothesize that not only the acoustic component of the frog's display is necessary to elicit foraging behaviour in the bat (Page and Ryan 2008), but that a close spatial relationship between the frog call and the water ripples is necessary to trigger a search-image in the bat. Female túngara frogs show perceptual binding of the acoustic and visual component of the male frog's display (Clark and Dukas 2003). Similarly, perceptual binding of acoustic and echo-acoustic cue might be present in the frog-eating bat, since the acoustic component imperatively precedes the ripple component in the wild.

I believe that the frog-eating bat and the túngara frog provide an outstanding system to get closer to answering the question whether FM bats can use water turbulences as foraging cues. With future research I want to address this subject in more detail. A related question that arose from my work regards the influence that the angle of ensonification has on the detection of water turbulences.

Influence of ensonification angle - In the spatial frequency detection experiment in Chapter 1, bats would have had the possibility to change the angle from which to echolocate at the targets. For instance, the set-up would have allowed them to choose a position directly above the discs or directly next to them. However, no such strategy could be observed. The typical position of a bat during the detection task was at 45° relative to the disc's centre at 40 cm distance. According to our impulse-response measurements in Chapter 1, this position yields the most conspicuous differences in target strength between the flat and the rippled disc. Impulse-response measurements were carried out with the speaker and microphone positioned at angles of 30°, 45°, 60° and 90° relative to the disc's centre at a distance of 40 cm to the disc. My data showed that echoes from rippled surfaces depend very much on sound impingement angle. For an ensonification angle of 45°, amplitude and duration of the impulse responses increased with increasing grating depth and spatial frequency. The difference in target strength between each rippled test disc and the flat reference disc paralleled the perceptual thresholds: target-strength differences (TSD) increased with increasing grating depth and spatial frequency, with a very sudden increase around the respective spatial frequency's grating-depth threshold. The discs' TSD at discrimination threshold for each spatial frequency roughly matches *P. discolor*'s threshold for TSD of about 5-7 dB (Heinrich et al. 2011). At a shallower ensonification angle of 30°, the relationship between TSD and spatial frequency/grating depth follows the same pattern as for the 45° ensonification, but shifted in a way that the TSDs at discrimination threshold were only 0-5 dB. This means that bats' detection thresholds would have been systematically worse had the bats ensonified the ripples from a shallower angle (if we assume that bats' spatial frequency sensitivity is indeed mediated by TSDs). At a steeper ensonification angle of 60°, the relationship between TSP and spatial frequency/grating depth becomes more erratic; for higher spatial frequencies, the sudden increase in TSD around the threshold grating depth is missing. At an ensonification angle of 90°, any relation between TSD and spatial frequency or grating depth is lost. It is noteworthy however, that from this vertical position all TSD values are 20 dB and higher, so well above *P. discolor*'s TSD threshold of 5-7 dB (Heinrich et al. 2011).

These results raise the question whether a flying bat also ensonifies the water surface at an angle of 45°. Rydell et al. (1999) report that *M. daubentonii* bats fly low over water and hold their heads horizontally, approximately in line with the body, during call emission. It was concluded that the echolocation beam is thus emitted parallel to the water surface. Sonar emissions are directional, i.e., the emission's sound level changes with its direction in azimuth and elevation. This spatial pattern has been quantified for several bat species (Surlykke et al. 2009; Brinklov et al. 2011; Jakobsen et al. 2013). It is generally referred to as the sonar beam, although this term is somewhat misleading because the echolocation beam is often lobe-shaped, not cone-shaped like a light beam.

When hunting in open air, the sonar beam of *M. daubentonii* can be described as circular (Jakobsen and Surlykke 2010). Their frequency-modulated call has most of its energy at 55 kHz. For this frequency, both the maximum horizontal and vertical beam width is approximately 120°. The sound pressure at 45° vertically off-axis is reduced by approximately 6 dB relative to the centre of the piston and by approximately 12 dB at 90° vertically off-axis, i.e., directly below the bat (Jakobsen and Surlykke 2010). A bat emitting its calls parallel to the water surface would therefore receive the strongest echoes from directly ahead, on-axis, but also audible echoes from the water surface ahead and directly below, including a 45° ensonification angle. However, as mentioned these sonar-beam measurements stem from *M. daubentonii* ensonifying an airborne target. Measuring beam size of bats flying above water is not a trivial task due to strong interference from water reflections. While horizontal beam-width of *M. daubentonii* when hunting over a water surface appears similar to when hunting in open air (Surlykke et al. 2009), to date no data are available for their vertical beam-width above water.

M. daubentonii emits its calls through the mouth. Both *P. discolor*, the species under study in this thesis, and *T. cirrhosus*, the frog-eating bat, belong to the family of Phyllostomid bats, which emit their echolocation calls through the nostrils. The nose leaf, name giving to this bat family, surrounds the nostrils. The nose leaf is a highly motile fleshy extension of the nose in the shape of a lancet. Phyllostomid bats produce sonar beams that are both highly directional (Brinklov et al. 2011; Surlykke et al. 2013) and highly dynamic (Linnenschmidt and Wiegrebe 2016), with the nose leaf determining especially the vertical directionality (Hartley and Suthers 1987; Vanderelst et al. 2010; Kugler and Wiegrebe 2017). The height of the sonar beam in Phyllostomid bats is much smaller than in *M. daubentonii*; around 60° in *Carollia perspicillata*, *P. discolor*, and *T. cirrhosus* (Brinklov et al. 2011; Linnenschmidt and Wiegrebe 2016; Surlykke et al. 2013). However, it is noteworthy that bats narrow their sonar beam in the confined environment of a laboratory in comparison to the wild (Surlykke et al. 2009) and possibly also when free-flying compared with when they are stationary (Brinklov et al. 2011).

Keeping all of these aspects in mind, we can conclude that an experiment with free-flying bats solving a spatial frequency detection task would give us more insight on the perception of rippled surfaces and its dependence on ensonification angle. Preceding the experiment in Chapter 1, I have tried to train free-flying *P. discolor* bats to the detection of rippled surfaces without success. While I can't exclude the possibility that they could have eventually learned this task, there are other bat species with foraging ecologies more suited to the task. Both *M. daubentonii* as a trawling mouth-emitting bat as well as *T. cirrhosus* as a nose-leaved bat make excellent candidates to study the question how bats optimize their calling angle on water surface ripples.

***Evaluation of transient cues* -** Prey-induced turbulences are temporary or transient cues, i.e., they change their reflective characteristics over time and eventually disappear. Bat echolocation faces special challenges with the analysis of transient cues: it is not continuous but stroboscopic. A bat calls, and then must be silent in order to hear the returning echo. In Chapter 2 and 3, I measured how small a periodic movement with a specific frequency can become to be still detectable by an echolocating bat. My results show that the sensitivity varies considerably among different temporal frequencies and that sensitivity depends on the quality of the cue that is modulated. In Chapter 2, I modulated echo delay and found a wagon-wheel effect that arises from interaction between the bat's call rate and the modulation rate. In this experiment, the wagon-wheel effect resulted in a steep drop in modulation sensitivity for modulation rates of 20 Hz and 50 Hz, rates comparable to the bats' own call rate within groups of emitted echolocation calls. One can ask whether a different call rate would then result in a wagon-wheel effect that emerges at different modulation rates. One can further ask what call rates do aerial hawking FM bats employ.

The call rates of insectivorous aerial hawking bats are often coupled to their own wing-beat rate. This strategy allows them to produce intense echolocation calls at low cost as a by-product of flight (Heblich 1986; Speakman and Racey 1991; Wong and Waters 2001). In particular, during the search phase these bats call only once every wing beat (Jones 1994; Kalko 1994). The resulting low call rates not only support perception of echoes with long delays, as they can be expected from distant prey items, they also prevent any wagon-wheel effect. Once an insect has been detected, the call rate increases: the closer the bat gets to its prey the earlier it receives the echo and emits the next call. During this approach phase the call rate gradually increases until the call series ends with the distinctive terminal buzz (Kalko and Schnitzler 1989). The ongoing increase in call rate would prevent the wagon-wheel effect and thus allow the bat to perceive the target's fluttering movement, enabling it to discriminate prey type and make appropriate foraging decisions. However, at higher call repetition rates during the approach phase, many bats of different families [e.g. Vespertilionidae, *M. nattereri* (Melcón et al. 2007), *E. fuscus* (Surlykke and Moss 2000); Phyllostomidae (Weinbeer et al. 2006); Rhinolophidae (Schnitzler 1968)] have been reported to keep their call rates stable for periods of up to 200 ms (Moss et al. 2006) before introducing a longer call pause. The longer intervals between these so called strobe-groups of calls have been suggested to serve as window for the bat to listen to echoes with longer delays or for functions such as integration of echo sequences and motor behaviour control (Wilson and Moss 2003). Moss and Surlykke (2010) assumed that bats adjust their call timing in response to perceptual demands. I suggest that the avoidance of a wagon-wheel effect within strobe-groups is one of those demands. In the virtual reality paradigm used in Chapters 2 and 3, a different simulated target distance may have lead to a change in the bats' call rate within strobe-groups. We would expect this to lead to different sensitivity for echo-delay modulation but not for echo-amplitude modulation; a hypothesis that remains to be tested.

Notably, it is unknown whether *P. discolor*, the species under study in this thesis, can forage on the wing. There is strong evidence that it forages on insects capable of flight (Willig et al. 1993), but we cannot know whether the bats catch this prey airborne or on the ground. We could shed light on this question with a simple experiment where *P. discolor* bats are given the chance to hunt airborne insects in a flight-room. Monitoring with stereo high-speed video recordings and with multi-channel ultrasound recordings would allow for flight-path and sonar-beam reconstruction. More information on *P. discolor's* foraging behavior will be advantageous in interpreting former and future scientific findings on this model species.

For aerial hawking bats, a failure to detect movement does not necessarily mean a failure to detect the prey altogether, but it might do so for trawling bats that rely on transient turbulences. The CF bat *Noctilio leporinus* can use echolocation to detect small water splashes or other cues produced when prey disrupt the water surface from below (Suthers 1965; Ubernickel et al. 2013). Can we expect similar sensitivity in FM bats? The prey-generated water ripples that the frog-eating bat *T. cirrhosus* exploits have a main temporal frequency of 12 Hz (Halfwerk et al. 2014). In Chapter 2 I found that in *P. discolor* the sensitivity to delay modulation at a rate of 10 Hz is fairly high. Given that *T. cirrhosus* uses a call rate similar to what we found during our experiments [25 Hz in cruising flight (Surlykke et al. 2013)], it should be well able to detect the ripples through the accompanying modulation of echo delays. In this case, a wagon-wheel effect is impossible, since the call rate is higher than the temporal frequency of the waves. Alternatively, Halfwerk et al. (2014) proposed that *T. cirrhosus* relies on the temporal variance in echo loudness for the detection of frog-generated ripples. They found that echoes returning from a rippled water surface had higher variance in amplitude than echoes from a smooth water surface. These results match our findings from Chapter 2, where the variation in echo amplitude between different phases of the amplitude modulation was very high at an amplitude-modulation rate of 10Hz.

We can conclude that dynamic cues changing at a slow rate, i.e., at least slower than the call emission rate of the bat, should be perceivable to FM bats both through the detection of changes in echo delay and in echo amplitude. Transient cues with higher temporal frequencies would very likely be subject to the wagon-wheel effect if a bat uses one fixed call rate. To find out more about how aerial hawking bats perceive transient cues, especially during the approach phase we require more experimental data on echolocation behaviour of free-flying FM bats. Stereotypical call grouping on the one hand may lead to a wagon-wheel effect, the longer inter-call intervals between call groups on the other hand may serve to prevent it.

Spatial frequency discrimination - In Chapter 1 I have shown that *P. discolor* is sensitive to spatial frequency. My experiment was limited to a detection task only. We can ask what are the capabilities of echolocating bats when it comes to the discrimination between different spatial frequencies? To test this experimentally, bats would be presented not with one flat and one rippled disc, but with two rippled discs differing in spatial frequency. Additionally, one could introduce high-frequency cues in the form of small prey items on top of different low-frequency backgrounds. I have attempted this experiment in the field with wild *M. daubentonii*, to no avail because the bats were not attracted to but rather avoided the experimental setup. A laboratory approach might yield more success. Furthermore, for the detection of spatial frequency, bats very likely use target strength cues. For the discrimination of two spatial frequencies with equal target strength, bats would be forced to abandon target-strength evaluation and most likely analyse temporal and/or spectral features of the echoes. My measurements have shown that the impulse responses of two discs with differing spatial frequency and grating depth (but equal target strength) differ in their temporal structure. Differences in echo temporal structure, “echo roughness”, are well perceptible to bats (Grunwald et al. 2004). Thus, these temporal envelope fluctuations may be an important cue for spatial-frequency discrimination. We propose that on top of target-strength analysis, FM bats rely on time-domain analyses of the complex echoes from wave surfaces to assess their spatial frequencies - another hypothesis that remains to be experimentally evaluated.

Prey detection performance over turbulent water – I have proposed the existence of a threshold degree of turbulence below which foraging efficiency is not affected because the spatial frequency content is below the cut-off frequency off the spatial high-pass filter. In order to test this hypothesis we designed a pilot experiment to quantify prey-detection performance above a real water body with tightly controlled distribution of both temporal and spatial frequencies (Figure D.2). Single wild bats (*Myotis daubentonii*) were captured and left to forage above an artificial pond in the flight laboratory. Prey items (live mealworms, larvae of the species *Tenebrio molitor*) floated on the water surface within a foraging patch measuring 110 x 120 cm. The bat’s behaviour was monitored with stereo high-speed video recordings and with stereo ultrasound recordings. I generated surface waves by means of four long-stroke bass speakers, whose membranes were connected to the water surface via Styrofoam cones. In order to create ripples of different frequency and amplitude, the speakers were driven with different stimuli (pink noise, low frequency pure tones) at different amplitude settings. The water surface was obliquely illuminated by red light from a slide projector and the resulting reflections of the waves were filmed off a projection screen erected behind the pond, together with the bat’s behaviour (Figure D.3). In the pilot data, I analysed the number of passes prior to a capture event as well as at the search time required to catch the prey item. A pass was defined as crossing the foraging area at low height (< 50cm). Search time was measured from the first pass to the first capture attempt. A successful capture attempt was defined by hearing a feeding buzz on the synchronized echolocation recordings emitted by the bat at the location of the prey.

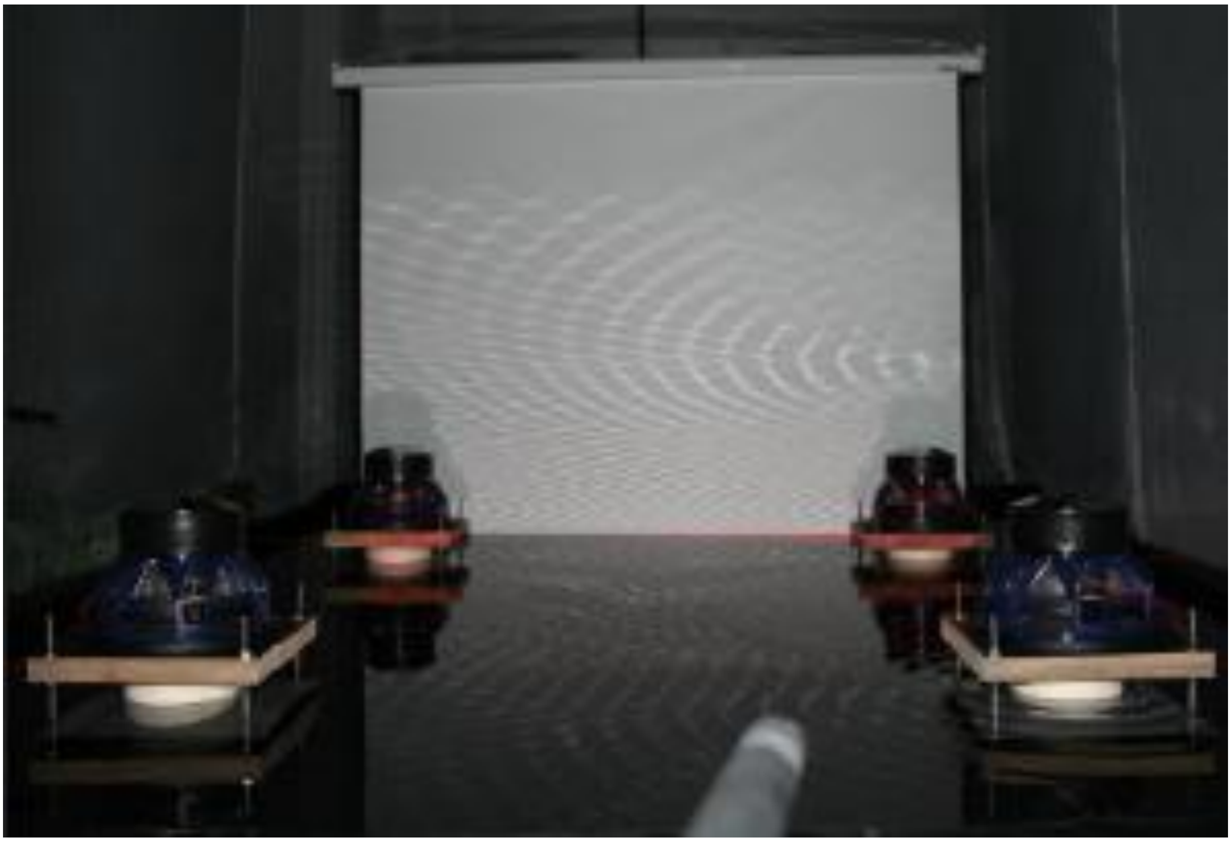

Figure D.2 Pilot setup for prey detection experiments. *Frontal view of the pool and the projection screen with reflections of the ripples that were created by the bass speakers. Note in the foreground inactive speaker on the left and active speaker on the right.*

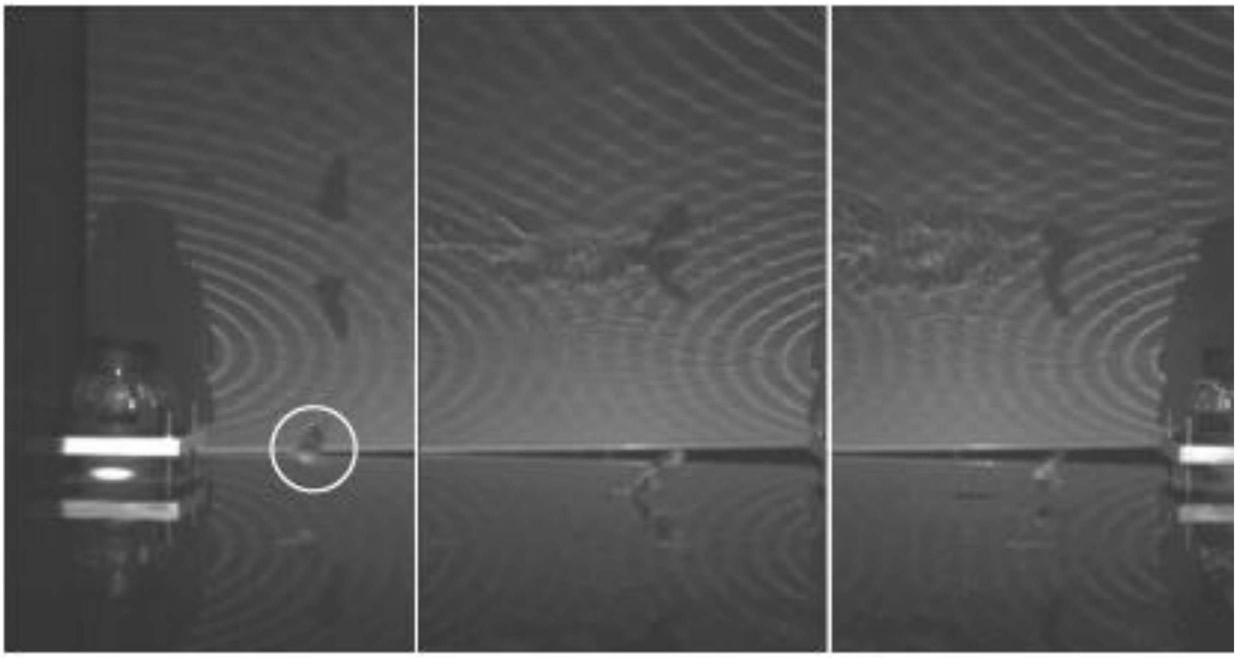

Figure D.3 Prey-capture success over turbulent water. *Still frames taken from high-speed footage show glimpses of a catching sequence: the bat (white circle) approaches the prey, catches it with its feet and rolls up in flight to take the prey out of the uropatagium.*

Bats reliably detected and localised prey floating on waves with a 5 Hz temporal frequency and a 17 m^{-1} spatial frequency. Failed capture attempts occurred in the sense that bats were not able to glean the prey off the surface or dropped it during further handling, but not due to erroneous localization. While these findings corroborate my conclusions from Chapter 1 that bats may rely on the segregation of high from low spatial frequencies for prey detection, the limited data set does not yet allow confirming or rejecting the hypothesis of an existing threshold for impairment caused by water turbulence.

Rydell et al. (1999) found that bats avoid ripples with amplitudes of 2-3 cm. Even at the highest amplitude setting, the waves in the pilot experiment did not reach this height. Additionally, S. Greif (personal communication) found that bats no longer recognize a water surface when it is covered with a coarsely meshed net. The net protruded much less from the water than the height of the waves generated in the pilot experiment, but the threads formed sharp edges, producing high spatial frequencies. Again, these observations indicate that bats rely on the segregation of high from low spatial frequencies for prey detection. The pilot setup allowed both the generation and documentation of the resulting spatio-temporal wave structure, but for further research both wave generation and monitoring need refined techniques: waves would be generated with horizontal rod actuators, driven by the existing speakers. Colouring the water black, and projecting a grid pattern vertically onto the water surface would improve documentation of the wave structure. The waves would perturb the grid pattern, and thus, filming the grid pattern from vertically above would allow reconstructing the 3D wave structure. An improved technical set-up will allow testing higher temporal and spatial frequencies.

Based on my results from the formal psychophysical detection experiments and the pilot data outlined above, we can devise some expectations: First, prey detection will likely become difficult for the trawling bats when the spatial frequency content of generated waves approaches the cut-off frequency of the spatial high-pass filter for this species. Second, prey detection will likely become difficult for the trawling bats when the spatial and temporal frequency content of generated waves approaches the frequency range of the local turbulence introduced by the floating prey item. Third, we can expect behavioural changes in flight and/or echolocation behaviour of the bats in such cases. Ultimately, these experiments will provide evidence in support of or against the existence of a turbulence threshold.

In this thesis, I have explored the capabilities and limitations of the echolocation system of frequency-modulating bats, mostly with regard to tasks that these bats face when they forage above water surfaces. My studies have answered many questions and brought up many new ones. In the same way, by writing these lines I close one door behind me and open many new doors ahead of me. What will guide me through all of these doors is the curiosity about what is going on in the brains of these small shadows that shoot swiftly through the warm air above a lake, on an early summer's evening.

REFERENCES

Altes RA (1995) Signal processing for target recognition in biosonar. Neural Netw 8 (7-8):1275-1295

Au WW, Suthers RA (2014) Production of biosonar signals: Structure and form. In: Biosonar. Springer, pp 61-105

Aubie B, Sayegh R, Faure PA (2012) Duration tuning across vertebrates. J Neurosci 32 (18):6373-6390

Bar M (2004) Visual objects in context. Nat Rev Neurosci 5 (8):617-629. doi:10.1038/nrn1476

Barclay R, Fenton M, Tuttle M, Ryan M (1981) Echolocation calls produced by *Trachops cirrhosus* (Chiroptera: Phyllostomatidae) while hunting for frogs. Can J Zool 59 (5):750-753

Beedholm K, Møhl B (1998) Bat sonar: an alternative interpretation of the 10-ns jitter result. J Comp Physiol A 182 (3):259-266. doi:10.1007/s003590050176

Bernal XE, Stanley Rand A, Ryan MJ (2007) Sexual differences in the behavioral response of túngara frogs, *Physalaemus pustulosus*, to cues associated with increased predation risk. Ethology 113 (8):755-763

Blauert J (1997) Spatial hearing: the psychophysics of human sound localization. MIT press,

Bleckmann H (1985) Perception of water surface waves: how surface waves are used for prey identification, prey localization, and intraspecific communication. In: Ottoson D (ed) Advances in Sensory Physiology, vol 5. Springer Verlag, Berlin, pp 147-166

Bleckmann H, Borchardt M, Horn P, Gorner P (1994) Stimulus discrimination and wave source localization in fishing spiders (*Dolomedes triton* and *D. okefinokensis*). J Comp Physiol A 174 (3):305-316

Bleckmann H, Rovner JS (1984) Sensory ecology of a semi-aquatic spider (*Dolomedes triton*). 1. Roles of vegetation and wind-generated waves in site selection. Behav Ecol Sociobiol 14 (4):297-301. doi:10.1007/bf00299501

Bleckmann H, Schwartz E (1982) The functional significance of frequency modulation within a wave train for prey localization in the surface-feeding fish *Aplocheilus lineatus* (Cyprinodontidae). Journal of Comparative Physiology 145 (3):331-339

Bleckmann H, Tittel G, Bluebaum-Gronau E (1989) The lateral line system of surface-feeding fish: Anatomy, physiology and behavior. Coombs, S., P. Goerner and H. Muenz.

Blood BR, Clark MK (1998) *Myotis vivesi*. Mammalian Species (588):1-5. doi:10.2307/3504455

Boonman AM, Boonman M, Bretschneider F, van de Grind WA (1998) Prey detection in trawling insectivorous bats: duckweed affects hunting behaviour in Daubenton's bat, *Myotis daubentonii*. Behav Ecol Sociobiol 44 (2):99-107

Borina F, Firzlaff U, Schuller G, Wiegrebe L (2008) Representation of echo roughness and its relationship to amplitude-modulation processing in the bat auditory midbrain. Eur J Neurosci 27 (10):2724-2732. doi:10.1111/j.1460-9568.2008.06247.x

Brinklov S, Jakobsen L, Ratcliffe JM, Kalko EKV, Surlykke A (2011) Echolocation call intensity and directionality in flying short-tailed fruit bats, *Carollia perspicillata* (Phyllostomidae). J Acoust Soc Am 129 (1):427-435. doi:10.1121/1.3519396

Britton ARC, Jones G, Rayner JMV, Boonman AM, Verboom B (1997) Flight performance, echolocation and foraging behaviour in pond bats, *Myotis dasycneme* (Chiroptera: Vespertilionidae). J Zool 241:503-522. doi:10.1111/j.1469-7998.1997.tb04842.x

Bullock TH, Grinnell AD, Ikezono E, Kameda K, Katsuki Y, Nomoto M, Sato O, Suga N, Yanagisawa K (1968) Electrophysiological studies of central auditory mechanisms in cetaceans. Z vergl Physiol 59 (2):117-156. doi:10.1007/bf00339347

Burdin V, Markov V, Reznik A, Skornyakov V, Chupakov A (1973) Determination of the just noticeable intensity difference for white noise in the Black Sea bottlenose dolphin (*Tursiops truncatus ponticus* Barabasch). Morphol Ecol Mar Mamm:169-173

Campbell FW, Robson JG (1968) Application of fourier analysis to the visibility of gratings. J Physiol 197 (3):551-566. doi:10.1113/jphysiol.1968.sp008574

Chiu C, Moss CF (2007) The role of the external ear in vertical sound localization in the free flying bat, *Eptesicus fuscus*. The Journal of the Acoustical Society of America 121 (4):2227-2235

Clark CW, Dukas R (2003) The behavioral ecology of a cognitive constraint: limited attention. Behav Ecol 14 (2):151-156

Condon CJ, White KR, Feng AS (1994) Processing of amplitude-modulated signals that mimic echoes from fluttering targets in the inferior colliculus of the little brown bat, *Myotis lucifugus*. J Neurophysiol 71 (2):768-784

Dankiewicz LA, Helweg DA, Moore PW, Zafran JM (2002) Discrimination of amplitude-modulated synthetic echo trains by an echolocating bottlenose dolphin. J Acoust Soc Am 112 (4):1702-1708. doi:10.1121/1.1504856

Dear SP, Fritz J, Haresign T, Ferragamo M, Simmons JA (1993) Tonotopic and functional organization in the auditory cortex of the big brown bat, *Eptesicus fuscus*. J Neurophysiol 70 (5):1988-2009. doi:10.1152/jn.1993.70.5.1988

Denzinger A, Schnitzler H-U (1994) Echo SPL influences the ranging performance of the big brown bat, *Eptesicus fuscus*. J Comp Physiol A 175 (5):563-571. doi:10.1007/bf00199477

Denzinger A, Schnitzler H-U (1998) Echo SPL, training experience, and experimental procedure influence the ranging performance in the big brown bat, Eptesicus fuscus. J Comp Physiol A 183 (2):213-224. doi:10.1007/s003590050249

Dickinson MH, Lent CM (1984) Feeding behavior of the medicinal leech, *Hirudo medicinalis* L. J Comp Physiol A 154 (4):449-455. doi:10.1007/bf00610160

Dijkgraaf S (1946) Die Sinneswelt der Fledermäuse. Experientia 2 (11):438-448

Dooling RJ, Searcy MH (1981) Amplitude modulation thresholds for the parakeet (*Melopsittacus undulatus*). J Comp Physiol A 143 (3):383-388

Enroth-Cugell C, Robson JG (1966) The contrast sensitivity of retinal ganglion cells of the cat. J Physiol 187 (3):517-552. doi:10.1113/jphysiol.1966.sp008107

Evans WE (1973) Echolocation by marine delphinids and one species of freshwater dolphin. J Acoust Soc Am 54:191-199

Falk B, Williams T, Aytekin M, Moss CF (2011) Adaptive behavior for texture discrimination by the free-flying big brown bat, *Eptesicus fuscus*. J Comp Physiol A 197 (5):491-503. doi:10.1007/s00359-010-0621-6

Fay RR, Wilber LA (1989) Hearing in vertebrates: a psychophysics databook. vol 86. Hill-Fay Associates, Winnetka, IL. doi:10.1121/1.398550

Fenton MB, Faure PA, Ratcliffe JM (2012) Evolution of high duty cycle echolocation in bats. J Exp Biol 215 (Pt 17):2935-2944. doi:10.1242/jeb.073171

Firzlaff U, Schörnich S, Hoffmann S, Schuller G, Wiegrebe L (2006) A neural correlate of stochastic echo imaging. J Neurosci 26 (3):785-791

Firzlaff U, Schuchmann M, Grunwald JE, Schuller G, Wiegrebe L (2007) Object-oriented echo perception and cortical representation in echolocating bats. PLoS Biol 5 (5):e100

Fremouw T, Faure PA, Casseday JH, Covey E (2005) Duration selectivity of neurons in the inferior colliculus of the big brown bat: tolerance to changes in sound level. J Neurophysiol 94 (3):1869-1878

Fukui D, Murakami M, Nakano S, Aoi T (2006) Effect of emergent aquatic insects on bat foraging in a riparian forest. J Anim Ecol 75 (6):1252-1258

Galambos R, Griffin DR (1942) Obstacle avoidance by flying bats: the cries of bats. J Exp Zool 89 (3):475-490

Geberl C, Brinkløv S, Wiegrebe L, Surlykke A (2015) Fast sensory–motor reactions in echolocating bats to sudden changes during the final buzz and prey intercept. Proc Natl Acad Sci USA 112 (13):4122-4127. doi:10.1073/pnas.1424457112

Geipel I, Jung K, Kalko EK (2013) Perception of silent and motionless prey on vegetation by echolocation in the gleaning bat *Micronycteris microtis*. Proceedings of the Royal Society B: Biological Sciences 280 (1754):20122830. doi:10.1098/rspb.2012.2830

Gellermann LW (1933) Chance orders of alternating stimuli in visual discrimination experiments. Ped Sem J Gen Psychol 42 (1):206-208

Ghose K, Moss CF (2003) The sonar beam pattern of a flying bat as it tracks tethered insects. J Acoust Soc Am 114 (2):1120-1131. doi:10.1121/1.1589754

Gibson G, Warren B, Russell IJ (2010) Humming in tune: sex and species recognition by mosquitoes on the wing. JARO 11 (4):527-540. doi:10.1007/s10162-010-0243-2

Goerlitz HR, Geberl C, Wiegrebe L (2010) Sonar detection of jittering real targets in a free-flying bat. J Acoust Soc Am 128 (3):1467-1475. doi:10.1121/1.3445784

Greiter W, Firzlaff U (2017) Echo-acoustic flow shapes object representation in spatially complex acoustic scenes. J Neurophysiol 117 (6):2113-2124

Griffin DR (1944) Echolocation by blind men, bats and radar. Science 100 (2609):589-590

Griffin DR (1958) Listening in the dark: The acoustic orientation of bats and men. Yale Univer. Press, Oxford, England

Griffin DR, Galambos R (1941) The sensory basis of obstacle avoidance by flying bats. J Exp Zool 86 (3):481-506

Griffin DR, Webster FA, Michael CR (1960) The echolocation of flying insects by bats. Anim Behav 8 (3):141-154. doi:doi.org/10.1016/0003-3472(60)90022-1

Grossetete A, Moss CF (1998) Target flutter rate discrimination by bats using frequency-modulated sonar sounds: Behavior and signal processing models. J Acoust Soc Am 103 (4):2167-2176

Grunwald JE, Schornich S, Wiegrebe L (2004) Classification of natural textures in echolocation. Proc Natl Acad Sci USA 101 (15):5670-5674

Gustafson Y, Schnitzler HU (1979) Echolocation and obstacle avoidance in the Hipposiderid bat *Asellia tridens*. Journal of Comparative Physiology 131 (2):161-167

Hagemann C, Esser K-H, Kössl M (2010) Chronotopically organized target-distance map in the auditory cortex of the short-tailed fruit bat. J Neurophysiol 103 (1):322-333. doi:10.1152/jn.00595.2009

Hahn WL (1908) Some habits and sensory adaptations of cave-inhabiting bats. Biol Bull 15 (3):135-164

Halfwerk W, Jones PL, Taylor RC, Ryan MJ, Page RA (2014) Risky ripples allow bats and frogs to eavesdrop on a multisensory sexual display. Science 343 (6169):413-416. doi:10.1126/science.1244812

Hartley DJ, Suthers RA (1987) The sound emission pattern and the acoustical role of the noseleaf in the echolocating bat, *Carollia perspicillata*. The Journal of the Acoustical Society of America 82 (6):1892-1900

Hartline HK (1949) Inhibition of activity of visual receptors by illuminating nearby retinal areas in the *Limulus* eye. Federation Proceedings 8 (1):69-69

Hartridge H (1920) The avoidance of objects by bats in their flight. J Physiol 54 (1-2):54-57

Heblich K (1986) Flügelschlag und Lautaussendung bei fliegenden und landenden Fledermäusen. Biona Report 5:139-156

Hechavarría JC, Macías S, Vater M, Voss C, Mora EC, Kössl M (2013) Blurry topography for precise target-distance computations in the auditory cortex of echolocating bats. Nat Commun 4:2587

Heinrich M, Warmbold A, Hoffmann S, Firzlaff U, Wiegrebe L (2011) The sonar aperture and its neural representation in bats. J Neurosci 31 (43):15618-15627. doi:10.1523/jneurosci.2600-11.2011

Heinrich M, Wiegrebe L (2013) Size constancy in bat biosonar? Perceptual interaction of object aperture and distance. PLoS One 8 (4):e61577. doi:10.1371/journal.pone.0061577

Hoffmann S, Firzlaff U, Radtke-Schuller S, Schwellnus B, Schuller G (2008) The auditory cortex of the bat *Phyllostomus discolor*: Localization and organization of basic response properties. BMCNeurosci 9:65

Hoffmann S, Genzel D, Prosch S, Baier L, Weser S, Wiegrebe L, Firzlaff U (2014) Biosonar navigation above water I: estimating flight height. J Neurophysiol 113 (4):1135-1145

Hoin-Radkovsky I, Bleckmann H, Schwartz E (1984) Determination of source distance in the surface-feeding fish *Pantodon buchholzi* (Pantodontidae) Anim Behav 32 (AUG):840-851. doi:10.1016/s0003-3472(84)80161-x

Holderied MW, von Helversen O (2006) Binaural echo disparity'as a potential indicator of object orientation and cue for object recognition in echolocating nectar-feeding bats. J Exp Biol 209 (17):3457-3468

Holland RA, Waters DA (2005) Echolocation signals and pinnae movement in the fruitbat *Rousettus aegyptiacus*. Acta Chiropt 7 (1):83-90. doi:10.3161/1733-5329(2005)7[83:esapmi]2.0.co;2

Jakobsen L, Hallam J, Moss CF, Hedenström A (2018) Directionality of nose-emitted echolocation calls from bats without a nose leaf (Plecotus auritus). J Exp Biol 221 (3):jeb171926

Jakobsen L, Ratcliffe JM, Surlykke A (2013) Convergent acoustic field of view in echolocating bats. Nature 493 (7430):93

Jakobsen L, Surlykke A (2010) Vespertilionid bats control the width of their biosonar sound beam dynamically during prey pursuit. Proceedings of the National Academy of Sciences 107 (31):13930-13935

Jen P, Suga N (1976) Coordinated activities of middle-ear and laryngeal muscles in echolocating bats. Science 191 (4230):950-952

Johnson CS (1967) Sound detection thresholds in marine mammals. Mar Bioacoustics 2:247-260

Jones G (1994) Scaling of wingbeat and echolocation pulse emission rates in bats: why are aerial insectivorous bats so small? Funct Ecol:450-457

Jones G, Rayner JMV (1991) Flight performance, foraging tactics and echolocation in the trawling insectivorous bat *Myotis adversus* (Chiroptera, Vespertilionidae) J Zool 225:393-412

Jones G, Rydell J (2003) Attack and Defense: Interactions between echolocating bats and their insect prey. In: Kunz TH, Fenton MB (eds) Bat Ecology. The University of Chicago Press, Chicago and London,

Kalko E (1994) Coupling of sound emission and wingbeat in naturally foraging European Pipistrelle Bats (Microchiroptera, Vespertilionidae). Folia Zoologica 43 (4):363-376

Kalko EK, Handley CO, Handley D (1996) Organization, diversity, and long-term dynamics of a Neotropical bat community. In: Long-term studies of vertebrate communities. Elsevier, pp 503-553

Kalko EKV (1990) Field study on the echolocation and hunting behaviour of the long-fingered bat, *Myotis capaccinii*. Bat Research News 31:42-43

Kalko EKV, Schnitzler HU (1989) The echolocation and hunting behavior of Daubenton's bat, *Myotis daubentonii*. Behav Ecol Sociobiol 24 (4):225-238

Kalko EKV, Schnitzler HU, Kaipf I, Grinnell AD (1998) Echolocation and foraging behavior of the lesser bulldog bat, *Noctilio albiventris*: preadaptations for piscivory? Behav Ecol Sociobiol 42 (5):305-319. doi:10.1007/s002650050443

Klymenko V, Weisstein N (1986) Spatial frequency differences can determine figure-ground organization. J Exp Psychol 12 (3):324-330. doi:10.1037//0096-1523.12.3.324

Kober R, Schnitzler HU (1990) Information in sonar echoes of fluttering insects available for echolocating bats. J Acoust Soc Am 87 (2):882-896. doi:10.1121/1.398898

Koselj K, Schnitzler HU, Siemers BM (2011) Horseshoe bats make adaptive prey-selection decisions, informed by echo cues. Proceedings of the Royal Society B: Biological Sciences 278 (1721):3034-3041. doi:10.1098/rspb.2010.2793

Kössl M, Vater M (1995) Cochlear structure and function in bats. In: Hearing by bats. Springer, pp 191-234

Kugler K, Wiegrebe L (2017) Echo-acoustic scanning with noseleaf and ears in phyllostomid bats. J Exp Biol 220 (15):2816

Kwiecinski GG (2006) Phyllostomus discolor. Mammalian Species 801:1-11

Lang HH (1980) Surface wave discrimination between prey and nonprey by the back swimmer *Notonecta glauca* L. (Hemiptera, Heteroptera). Behav Ecol Sociobiol 6 (3):233-246. doi:10.1007/bf00569205

Lawrence BD, Simmons JA (1982) Echolocation in bats - the external ear and perception of the vertical positions of targets. Science 218 (4571):481-483

Linnenschmidt M, Wiegrebe L (2016) Sonar beam dynamics in leaf-nosed bats. Sci Rep 6:29222. doi:10.1038/srep29222

Ma J, Jones G, Zhang S, Shen J, Metzner W, Zhang L, Liang B (2003) Dietary analysis confirms that Rickett's big-footed bat (*Myotis ricketti*) is a piscivore. J Zool 261 (3):245-248

Mackey RL, Barclay RMR (1989) The influence of physical clutter and noise on the activity of bats over water. Can J Zool 67 (5):1167-1170

Masters W, Raver K (1996) The degradation of distance discrimination in big brown bats (*Eptesicus fuscus*) caused by different interference signals. J Comp Physiol A 179 (5):703-713

Masters WM, Jacobs SC (1989) Target detection and range resolution by the big brown bat (*Eptesicus fuscus*) using normal and time-reversed model echoes. J Comp Physiol A 166 (1):65-73. doi:10.1007/bf00190211

Maxim H (1912) Preventing Collisions at Sea. Sci Am 107:80-81

Measor K, Yarrow S, Razak KA (2018) Topography of sound level representation in the FM sweep selective region of the pallid bat auditory cortex. Hearing Res

Melcón ML, Denzinger A, Schnitzler H-U (2007) Aerial hawking and landing: approach behaviour in Natterer's bats, *Myotis nattereri* (Kuhl 1818). J Exp Biol 210 (24):4457-4464

Menne D, Kaipf I, Wagner I, Ostwald J, Schnitzler HU (1989) Range estimation by echolocation in the bat *Eptesicus fuscus*: Trading of phase versus time cues. J Acoust Soc Am 85 (6):2642-2650. doi:10.1121/1.397758

Michelson AA (1927) Studies in Optics. University of Chicago Press,

Miller LA (1991) Arctiid moth clicks can degrade the accuracy of range difference discrimination in echolocating big brown bats, *Eptesicus fuscus*. J Comp Physiol A 168 (5):571-579. doi:10.1007/bf00215079

Mogdans J, Ostwald J, Schnitzler HU (1988) The role of pinna movement for the localization of vertical and horizontal wire obstacles in the greater horseshoe bat, *Rhinolophus ferrumequinum*. J Acoust Soc Am 84 (5):1676-1679. doi:10.1121/1.397183

Moore BC (2012) An introduction to the psychology of hearing. Brill,

Moore PW, Pawloski DA, Dankiewicz L (1995) Interaural time and intensity difference thresholds in the bottlenose dolphin (*Tursiops truncatus*). Sens Syst Aqu Mamm:11-23

Morinaka Y, Hosokawa T, Hagino T, Hiryu S, Riquimaroux H, Watanabe Y (2007) A sound localization model based on the alternate pinna movement of CF-FM bats. Sci Eng Rev Doshisha Univ 48 (2):63-69

Moss CF, Bohn K, Gilkenson H, Surlykke A (2006) Active listening for spatial orientation in a complex auditory scene. PLoS Biol 4 (4):e79

Moss CF, Schnitzler H-U (1989) Accuracy of target ranging in echolocating bats: acoustic information processing. J Comp Physiol A 165 (3):383-393

Moss CF, Surlykke A (2010) Probing the natural scene by echolocation in bats. Front Behav Neurosci 4. doi:10.3389/fnbeh.2010.00033

Moss CF, Zagaeski M (1994) Acoustic information available to bats using frequency-modulated sounds for the perception of insect prey. J Acoust Soc Am 95 (5):2745-2756. doi:10.1121/1.409843

Neuweiler G (1984) Foraging, echolocation and audition in bats. Naturwissenschaften 71 (9):446-455

Neuweiler G (1990) Auditory adaptations for prey capture in echolocating bats. Physiol Rev 70 (3):615-641

Neuweiler G (1993) Biologie der Fledermäuse. Thieme,

Novick A, Griffin DR (1961) Laryngeal mechanisms in bats for the production of orientation sounds. J Exp Zool 148 (2):125-145

Nowak RM (1994) Walker's bats of the world. JHU Press,

O'Neill W, Suga N (1979) Target range-sensitive neurons in the auditory cortex of the mustache bat. Science 203 (4375):69-73. doi:10.1126/science.758681

Ostwald J, Schnitzler H-U, Schuller G (1988) Target discrimination and target classification in echolocating bats. In: Nachtigall PE, Moore PWB (eds) Animal Sonar: Processes and Performance. Springer US, Boston, MA, pp 413-434

Page RA, Ryan MJ (2008) The effect of signal complexity on localization performance in bats that localize frog calls. Anim Behav 76 (3):761-769

Pierce G, Griffin DR (1938) Experimental determination of supersonic notes emitted by bats. J Mammal 19 (4):454-455

Pringle J (1949) The excitation and contraction of the flight muscles of insects. J Physiol 108 (2):226-232

Ratcliffe JM, Elemans CP, Jakobsen L, Surlykke A (2013) How the bat got its buzz. Biol Lett 9 (2):20121031

Rayleigh L (1907) XII. On our perception of sound direction. The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science 13 (74):214-232

Roeder KD (1963) Echoes of ultrasonic pulses from flying moths. Biol Bull 124 (2):200-210. doi:10.2307/1539496

Rother G, Schmidt U (1982) The influence of visual information on echolocation in *Phyllostomus discolor* (Chiroptera). Z Säugetierkunde 47 (6):324-334

Roverud RC, Grinnell AD (1985) Discrimination performance and echolocation signal integration requirements for target detection and distance determination in the CF/FM bat, *Noctilio albiventris*. J Comp Physiol A 156 (4):447-456

Roverud RC, Nitsche V, Neuweiler G (1991) Discrimination of wingbeat motion by bats, correlated with echolocation sound pattern. J Comp Physiol A 168 (2):259-263

Ruczynski I, Kalko EK, Siemers BM (2007) The sensory basis of roost finding in a forest bat, *Nyctalus noctula*. J Exp Biol 210 (Pt 20):3607-3615. doi:10.1242/jeb.009837

Ruedi M, Mayer F (2001) Molecular Systematics of Bats of the Genus *Myotis* (Vespertilionidae) Suggests Deterministic Ecomorphological Convergences. Mol Phylogen Evol 21 (3):436-448

Ryan MJ (1980) Female mate choice in a neotropical frog. Science 209 (4455):523-525

Rydell J, Miller LA, Jensen ME (1999) Echolocation constraints of Daubenton' s bat foraging over water. Funct Ecol 13 (2):247-255

Salvi RJ, Giraudi DM, Henderson D, Hamernik RP (1982) Detection of sinusoidally amplitude modulated noise by the chinchilla. J Acoust Soc Am 71 (2):424-429. doi:10.1121/1.387445

Sanderson MI, Simmons JA (2000) Neural responses to overlapping FM sounds in the inferior colliculus of echolocating bats. J Neurophysiol 83 (4):1840-1855

Schmidt S (1988a) Discrimination of target surface structure in the echolocating bat, *Megaderma lyra*. In: Nachtigall PE, Moore PWB (eds) Animal Sonar: Processes and Performance. Springer US, Boston, MA, pp 507-511. doi:10.1007/978-1-4684-7493-0_52

Schmidt S (1988b) Evidence for a spectral basis of texture perception in bat sonar. Nature 331 (6157):617

Schmieder DA, Kingston T, Hashim R, Siemers BM (2010) Breaking the trade-off: rainforest bats maximize bandwidth and repetition rate of echolocation calls as they approach prey. Biol Lett 6 (5):604-609. doi:10.1098/rsbl.2010.0114

Schnitzler H-U, Hackbarth H, Heilmann U, Herbert H (1985) Echolocation behavior of rufous horseshoe bats hunting for insects in the flycatcher-style. J Comp Physiol A 157 (1):39-46. doi:10.1007/bf00611093

Schnitzler HU (1968) Die Ultraschall-Ortungslaute der Hufeisen-Fledermäuse (Chiroptera-Rhinolophidae) in verschiedenen Orientierungssituationen. Z vergl Physiol 57:376-408

Schnitzler HU, Flieger E (1983) Detection of oscillating target movements by echolocation in the Greater Horsehoe bat. Journal of Comparative Physiology 153 (3):385-391

Schnitzler HU, Kalko EKV (2001) Echolocation by insect-eating bats. Bioscience 51 (7):557-569

Schnitzler HU, Kalko EKV, Kaipf I, Grinnell AD (1994) Fishing and echolocation behavior of the Greater Bulldog bat, *Noctilio leporinus*, in the field. Behav Ecol Sociobiol 35 (5):327-345. doi:10.1007/bf00184422

Schoernich S, Wiegrebe L (2008) Phase sensitivity in bat sonar revisited. J Comp Physiol A 194 (1):61-67. doi:10.1007/s00359-007-0290-2

Schondube JE, Herrera-M LG, Martínez del Rio C (2001) Diet and the evolution of digestion and renal function in phyllostomid bats. Zoology 104 (1):59-73. doi:doi.org/10.1078/0944-2006-00007

Schuller G (1979) Coding of small sinusoidal frequency and amplitude modulations in the inferior colliculus of 'CF-FM'bat, *Rhinolophus ferrumequinum*. Exp Brain Res 34 (1):117-132

Seibert A-M, Koblitz JC, Denzinger A, Schnitzler H-U (2015) Bidirectional echolocation in the bat *Barbastella barbastellus*: different signals of low source level are emitted upward through the nose and downward through the mouth. PLoS One 10 (9):e0135590

Sekuler R, Blake R (1985) Perception. Kopf NY, New York

Siemers BM, Baur E, Schnitzler HU (2005) Acoustic mirror effect increases prey detection distance in trawling bats. Naturwissenschaften 92 (6):272-276. doi:10.1007/s00114-005-0622-4

Siemers BM, Dietz C, Nill D, Schnitzler HU (2001a) *Myotis daubentonii* is able to catch small fish. Acta Chiropt 3 (1):71-75

Siemers BM, Schnitzler HU (2004) Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. Nature 429 (6992):657-661

Siemers BM, Stilz P, Schnitzler HU (2001b) The acoustic advantage of hunting at low heights above water: behavioural experiments on the European 'trawling' bats *Myotis capaccinii*, *M. dasycneme* and *M. daubentonii*. J Exp Biol 204 (22):3843-3854

Simmons J, Kick S, Lawrence B, Hale C, Bard C, Escudie B (1983) Acuity of horizontal angle discrimination by the echolocating bat, *Eptesicus fuscus*. J Comp Physiol A 153 (3):321-330

Simmons J, Lavender W, Lavender B, Doroshow C, Kiefer S, Livingston R, Scallet A, Crowley D (1974) Target structure and echo spectral discrimination by echolocating bats. Science 186 (4169):1130-1132

Simmons JA (1971) Echolocation in bats - Signal processing of echoes for target range. Science 171 (3974):925-&. doi:10.1126/science.171.3974.925

Simmons JA (1973) The resolution of target range by echolocating bats. J Acoust Soc Am 54 (1):157-173. doi:10.1121/1.1913559

Simmons JA (1979) Perception of echo phase information in bat biosonar. Science 204 (4399):1336-1338. doi:10.1126/science.451543

Simmons JA, Fenton MB, Ofarrell MJ (1979) Echolocation and pursuit of prey by bats Science 203 (4375):16-21. doi:10.1126/science.758674

Simmons JA, Ferragamo M, Moss CF, Stevenson SB, Altes RA (1990) Discrimination of jittered sonar echoes by the echolocating bat, *Eptesicus fuscus* - The shape of target images in echolocation. J Comp Physiol A 167 (5):589-616

Simmons JA, Ferragamo MJ, Sanderson MI (2003) Echo delay versus spectral cues for temporal hyperacuity in the big brown bat, *Eptesicus fuscus*. J Comp Physiol A 189 (9):693-702. doi:10.1007/s00359-003-0444-9

Simmons JA, Houser D, Kloepper L (2014) Localization and classification of targets by echolocating bats and dolphins. In: Surlykke A, Nachtigall PE, Fay RR, Popper AN (eds) Biosonar. Springer New York, New York, NY, pp 169-193. doi:10.1007/978-1-4614-9146-0_6

Simmons JA, Neretti N, Intrator N, Altes RA, Ferragamo MJ, Sanderson MI (2004) Delay accuracy in bat sonar is related to the reciprocal of normalized echo bandwidth, or Q. Proc Natl Acad Sci USA 101 (10):3638-3643

Sotavalta O (1953) Recordings of high wing-stroke and thoracic vibration frequency in some midges. Biol Bull 104 (3):439-444

Speakman J, Racey P (1991) No cost of echolocation for bats in flight. Nature 350 (6317):421

Speakman JR (2001) The evolution of flight and echolocation in bats: another leap in the dark. Mamm Rev 31 (2):111-130

Sum YW, Menne D (1988) Discrimination of fluttering targets by the FM-bat *Pipistrellus stenopterus*. J Comp Physiol A 163 (3):349-354. doi:10.1007/bf00604010

Surlykke A, Jakobsen L, Kalko EK, Page RA (2013) Echolocation intensity and directionality of perching and flying fringe-lipped bats, *Trachops cirrhosus* (Phyllostomidae). Front Physiol 4:143

Surlykke A, Miller LA (1985) The influence of arctiid moth clicks on bat echolocation; jamming or warning? J Comp Physiol A 156 (6):831-843. doi:10.1007/bf00610835

Surlykke A, Moss CF (2000) Echolocation behavior of big brown bats, *Eptesicus fuscus,* in the field and the laboratory. The Journal of the Acoustical Society of America 108 (5):2419-2429

Surlykke A, Pedersen SB, Jakobsen L (2009) Echolocating bats emit a highly directional sonar sound beam in the field. Proc R Soc B 276 (1658):853-860. doi:10.1098/rspb.2008.1505

Suthers RA (1965) Acoustic orientation by fish-catching bats. J Exp Zool 158 (3):319-347

Thompson D, Fenton M (1982) Echolocation and feeding behaviour of *Myotis adversus* (Chiroptera: Vespertilionidae). Aust J Zool 30 (4):543-546

Tian B, Schnitzler HU (1997) Echolocation signals of the Greater Horseshoe bat (Rhinolophus ferrumequinum) in transfer flight and during landing. J Acoust Soc Am 101 (4):2347-2364. doi:10.1121/1.418272

Ubernickel K, Tschapka M, Kalko EK (2013) Flexible echolocation behavior of trawling bats during approach of continuous or transient prey cues. Front Physiol 4:96. doi:10.3389/fphys.2013.00096

Vanderelst D, De Mey F, Peremans H, Geipel I, Kalko E, Firzlaff U (2010) What noseleaves do for FM bats depends on their degree of sensorial specialization. PLoS One 5 (8):e11893. doi:10.1371/journal.pone.0011893

Vanderplank FL (1950) Air-speed/wing-tip speed ratios of insect flight. Nature 165:806. doi:10.1038/165806a0

Veselka N, McErlain DD, Holdsworth DW, Eger JL, Chhem RK, Mason MJ, Brain KL, Faure PA, Fenton MB (2010) A bony connection signals laryngeal echolocation in bats. Nature 463 (7283):939

Viemeister NF (1979) Temporal modulation transfer functions based upon modulation thresholds. J Acoust Soc Am 66 (5):1364-1380. doi:10.1121/1.383531

Von Bekesy G (2017) Sensory Inhibition. Princeton University Press,

Von Der Emde G, Menne D (1989) Discrimination of insect wingbeat frequencies by the bat *Rhinolophus ferrumequinum*. J Comp Physiol A 164 (5):663-672

Von der Emde G, Schnitzler HU (1986) Fluttering target detection in Hipposiderid bats J Comp Physiol A 159 (6):765-772

Von der Emde G, Schnitzler HU (1990) Classification of insects by echolocating Greater Horseshoe bats. J Comp Physiol A 167 (3):423-430

Von Frenckell B, Barclay RMR (1987) Bat activity over calm and turbulent water. Can J Zool 65 (2):219-222

Warren RD, Waters DA, Altringham JD, Bullock DJ (2000) The distribution of Daubenton's bats (*Myotis daubentonii*) and pipistrelle bats (*Pipistrellus pipistrellus*) (Vespertilionidae) in relation to small-scale variation in riverine habitat. Biological Conservation 92 (1):85-91

Weinbeer M, Meyer CFJ, Kalko EKV (2006) Activity pattern of the trawling phyllostomid bat, *Macrophyllum macrophyllum*, in Panamá. Biotropica 38 (1):69-76. doi:10.1111/j.1744-7429.2006.00101.x

Weissenbacher P, Wiegrebe L (2003) Classification of virtual objects in the echolocating bat, *Megaderma lyra*. Behav Neurosci 117 (4):833-839. doi:10.1037/0735-7044.117.4.833

Wiesel TN, Hubel DH (1966) Spatial and chromatic interactions in the lateral geniculate body of the rhesus monkey. J Neurophysiol 29 (6):1115-&

Willig MR, Camilo GR, Noble SJ (1993) Dietary overlap in frugivorous and insectivorous bats from edaphic cerrado habitats of Brazil. J Mammal 74 (1):117-128

Wilson WW, Moss CF (2003) Sensory-motor behavior of free-flying FM bats during target capture. In: Thomas JA, Moss CF, Vater M (eds) Advances in the Study of Echolocation in Bats and Dolphins. Chicago University Press, Chicago, Wohlgemuth MJ, Luo J, Moss CF (2016) Three-dimensional auditory localization in the echolocating bat. Curr Opin Neurobiol 41:78-86. doi:https://doi.org/10.1016/j.conb.2016.08.002

Wong J, Waters D (2001) The synchronisation of signal emission with wingbeat during the approach phase in soprano pipistrelles (*Pipistrellus pygmaeus*). J Exp Biol 204 (3):575-583

Wotton J, Simmons J (2000) Spectral cues and perception of the vertical position of targets by the big brown bat, *Eptesicus fuscus*. The Journal of the Acoustical Society of America 107 (2):1034-1041

Zsebők S, Kroll F, Heinrich M, Genzel D, Siemers BM, Wiegrebe L (2013) Trawling bats exploit an echo-acoustic ground effect. Front Physiol 4:65-65. doi:10.3389/fphys.2013.00065

TABLE OF FIGURES

INTRODUCTION

CHAPTER 1

CHAPTER 2

CHAPTER 3

Discussion

AUTHOR CONTRIBUTIONS

Chapter 1

ALB, LW and HRG conceived and designed the study. ALB performed the experiments. ALB, LW and HRG analysed the data. ALB and LW wrote the manuscript, ALB and HRG revised it. LW and HRG supervised the study and are co-senior authors on this work.

Chapter 2

ALB and LW conceived and designed the study. ALB performed the experiment. ALB and LW analysed the data and wrote the manuscript.

Chapter 3

ALB and LW conceived and designed the study. KJS performed the experiment. ALB and LW analysed the data. ALB wrote the manuscript with assistance from LW. All authors discussed the analysis and interpretation of the results.

Notes:

ALB	Leonie Baier
HRG	Holger Görlitz
KJS	Kristin Stelzer
LW	Lutz Wiegrebe

LIST OF PUBLICATIONS

1. ***Baier AL****, Wiegrebe L, and Goerlitz HR:* Bat echo-imaging perceives spatial frequency with a non-spatial sensor*. submitted*

2. ***Baier AL*** *and Wiegrebe L:* Flutter sensitivity in FM bats. Part I: delay modulation. Journal of Comparative Physiology A (2018) *in press* https://doi.org/10.1007/s00359-018-1291-z

3. ***Baier AL****, Stelzer K-J, and Wiegrebe L:* Flutter sensitivity in FM bats. Part II: amplitude modulation. Journal of Comparative Physiology A (2018) *in press* https://doi.org/10.1007/s00359-018-1292-y

4. *Hoffmann S, Genzel D, Prosch S,* **Baier L***, Weser S, Wiegrebe L, and Firzlaff U:* Biosonar navigation above water I: estimating flight height. Journal of Neurophysiology 113(4): 1135-1145 (2015)

5. *Voigt CC,* ***Baier L****, Speakman JR, and Siemers BM*: Stable carbon isotopes in exhaled breath as tracers for dietary information in birds and mammals. Journal of Experimental Biology, 211: 2233-2238 (2008)

6. **Baier L***, Hoffmann S*, Borina F*, Schuller G, Wiegrebe L, and Firzlaff U: Psychophysical and neurophysiological hearing thresholds in the bat Phyllostomus discolor. Journal of Comparative Physiology A, 194: 39-47 (2008) *co-first authors (order changed for emphasis)

Acknowledgements

This journey began many years ago, one (probably rainy) autumn day in 2003, at lunchtime. Over his plate of cafeteria food, Lutz Wiegrebe mentioned he was going to start a project on object scaling in bat echolocation. He was looking for somebody to turn this into a Diploma thesis. I was indeed interested but dismissed the thought because I was just a few weeks into my 5th semester and literally years away from writing the thesis. I was doing a practical on bats in the basement of Luisenstraße 14, the old Zoological Institute. But the weeks went by and I kept thinking about that project. So one day in December I went and asked Lutz about it. From that day on, the bats have been a part of my life. Three years later I earned my Diploma, my first real job already lined up: I will never forget how I first met Björn Siemers. The day of my interview, I entered his then makeshift office in Seewiesen and before I could open my mouth he was already beaming at me, offering cookies and later the job. Another six years later, Björn had passed away, leaving all of us devastated, and with an uncertain future. Lutz not only accepted the post of interim group leader, but also me as his PhD student, brightening the future a great deal. Holger Görlitz, who had only recently joined Björn’s lab as post-doc, prepared to take over and agreed to supervise my PhD work in Seewiesen.

To these three people, Lutz, Björn and Holger, I am indefinitely grateful. They have shaped my scientific and therefore also my personal life. Lutz, thank you for constantly nudging me gently (and sometimes not so gently) in the right direction. I wouldn’t have arrived at this point without you. Björn, thank you for teaching me the passion for science and for life. Holger, thank you for your continuous earnest help and for being an example in so many ways.

I also want to offer my thanks to all the people in the different labs that I have worked with over all those years. With some of them, ways have parted, others I'm still lucky to have in my life. You know who you are.

The Wiegrebe lab then and now: Andrea Lingner, Daria Genzel, Ella Lattenkamp, Felizia Czech, Jinhong Luo, Kathrin Kugler, Kristin Stelzer, Maike Schuchmann, Marga Überfuhr, Markus Drexl, Meike Linnenschmidt, Melina Heinrich, Michael Schutte, Peter Wagenhäuser, Stephanie Shields, Sven Schörnich, and Vanessa Kratzer. Other LMU crewmembers: Grothes Löwenzähne, Sabrina Schenk, Barbara Eißner, Miscu Pecka, Steffen Katzner, Kathrin Koros and Sera Camyesil. In addition, I thank Benedikt Grothe and the LMU for providing outstanding research infrastructure.

In Seewiesen then and now: Adriana Dorado, Antoniya Hubancheva, Daniel Lewanzik, Daniela Schmieder, Diana Werner, Dylan Gomes, Frauke Krüger, Ilse

Bayer, Irek Ruczynski, Ivo Borissov, Jinhong Luo, Kathrin Kugler, Klemen Koselj, Maike Schuchmann, Markus Clarin, Melina Heinrich, Nicole Drenkard, Rachel Page, Renate Heckel-Merz, Sándor Zsebők, Samuel Kaiser, Sara Troxell, Sébastien Puechmaille, Sophie von Merten, Stefan Greif, Tess Driessens, Thejasvi Beleyur, Theresa Clarin, Theresa Hügel, and Vincent van Meir. Other "roost mates" at the MPIO: Jaap van Schaik, Laurie O'Neill, Henrik Brumm, Nine Spehn, Sue Ann Zollinger, Alexander Krikellis, Kristina Schmid, Uschi Ludwig, Erich Koch, Klaus Pichler, and Christian Völk. Above all, I thank the directors, Manfred Gahr and Bart Kempenaers, and Thomas Dzionsko for keeping us up in the air to this day.

From all the people in Gamboa, my thanks go first and foremost to Rachel Page, for giving me the opportunity to come to this magical place. The batlab team made my stay there unforgettable, instructive, and rewarding: Ahana Fernandez, Basti Stockmaier, Claire Hemingway, Darija Josić, Devin Herlihy, Erin Swerdfeger, Gerry Carter, Gloria Gessinger, Gregg Cohen, Hugo Loning, Hugo Narizano, Inga Geipel, Ivon Vassileva, Jineth Berrío, Jo Kalbantner, Judith Smit, Kirk Silas, Krista Patriquin, Lauren Meckler, May Dixon, Michelle Nowak, Mitchel McCloskey, Nora Serve, Olivia Feagles, Rachel Crisp, Samuel Kaiser, Tate Ackerman. Adriana Bilgray and Aristoteles Villegas I thank for administrative and organisational support. My roommate Sara Fern Leitman I thank for her wonderful crazy companionship, literally day or night.

At the VU in Amsterdam I thank Wouter Halfwerk for his continued advice and support as well as Ralph Simon and Estefania Velilla for all their help and insight. More thanks to my favourite roomie David Hamilton for our binge-watching/pizza/ice-cream nights.

I am grateful to Dietmar Nill for his marvellous bat photographs.

I thank all my funding agencies, first and foremost the German Research Foundation (Deutsche Forschungsgemeinschaft), the German Academic Exchange Service (Deutscher Akademischer Austauschdienst), the Company of Biologists, the Smithsonian Institution, and the International Max Planck Research School.

My special thanks go to Mäggi Hieber and Evi Fricke from the IMPRS for their wonderful continued support.

I want to thank Dina Dechmann, Kamran Safi, Wolfgang Goymann, and George Pollak for their counsel in scientific writing.

Kristin Stelzer has earned a special place in this thesis. Thank you for your extraordinary stamina and enthusiasm and all your wonderful stories!

My thanks go also to Prof. Herbert Peremans in Antwerpen for taking the time to come to Munich for my PAC meetings, for helpful discussions and suggestions, and for a fun Zipflbob-race!

I thank my fantastic friend Heather Mayberry, who has always been there to help me tackle the "Challenges of a PhD".

Lastly, I thank my family. My parents, grandparents and sisters have always supported my decisions, from near and afar. Thank you for being my hidden reserve.