Perception of Echo-Acoustic Flow in Bats

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Zusammenfassung

Fledermäuse sind nachtaktive Tiere, die sich echoortend schnell und geschickt in der Dunkelheit fortbewegen. Sie senden Echoortungsrufe im Ultraschallbereich aus, die von naheliegenden Objekten zurückgeworfen werden. Durch die Analyse von Unterschieden zwischen ausgesandtem Ruf und zurückkommendem Echo können Fledermäuse Informationen über Form, Größe, Ausrichtung, Oberflächenstruktur und den dreidimensionalen Standort von Objekten erlangen. Im Flug bewegt sich die Fledermaus relativ zur Umgebung und erfährt folglich einen Fluss von sensorischer Information von den diversen naheliegenden Objekten.

Diese kumulative Dissertation umfasst zwei eigenständige Studien zur Wahrnehmung von echo-akustischem Fluss bei phyllostomiden Fledermäusen. Der erste Teil der Dissertation befasst sich mit der Frage, ob Fledermäuse echo-akustischen Fluss zur Flugsteuerung nutzen. Für Tiere, die sich visuell orientieren, ist optischer Fluss ein wichtiger Parameter, der es erlaubt die relative Position zur Umwelt abzuschätzen, und der zur Bewegungssteuerung herangezogen wird. Im ersten Experiment testete ich, ob echo-akustischer Fluss den Flug von Fledermäusen durch enge Passagen beeinflusst. Dazu dressierte ich Fledermäuse einen engen Korridor mit strukturierten Seitenwänden zu durchfliegen. In der Tat veränderten die Fledermäuse ihre Flugpfade entsprechend der Stärke des echo-akustischen Flusses, der durch die Seitenwände hervorgerufen wurde: die Fledermäuse zentrierten die Flugbahn um die Mittellinie, wenn beide Seitenwände gleich starken echo-akustischen Fluss hervorriefen und verlagerten die Flugpfade auf die Seite, die schwächeren echo-akustischen Fluss hervorrief, wenn dieser sich zwischen den beiden Seiten unterschied. Diese Flugpfadanpassungen entsprechen den Flugpfadanpassungen visuell orientierter Flieger (Vögel, Fluginsekten). In Anbetracht der Tatsache, dass, anders als im Sehen, in der Echoortung über das Echo Delay (den Laufzeitunterschied zwischen Echoortungsruf und Echo) direkte Distanzinformation zur Verfügung steht, ist diese Erkenntnis überraschend. Trotzdem nehmen Fledermäuse Seitenwände, die schwächeren oder stärkeren echo-akustischen Fluss hervorrufen, als unterschiedlich wichtig wahr. Des Weiteren habe ich analysiert, ob Fledermäuse Anpassungen hinsichtlich des Flug- und Echoortungsverhaltens an echo-akustischen Fluss zeigen. Anders als visuell orientierte Flieger

passten die Fledermäuse die Fluggeschwindigkeit nicht an den echo-akustischen Fluss an. Echo-akustischer Fluss hatte einen schwachen Effekt auf die Beschallungsrate und das zeitliche Beschallungsmuster, ein Hinweis darauf, dass die Fledermäuse die Strategie, wie sie die Umgebung echo-akustisch erforschen, nur in geringem Maße an die Stärke des echo-akustischen Flusses anpassen. Insgesamt zeigen die Ergebnisse, dass echo-akustischer Fluss von echoortenden Fledermäusen zur Flugsteuerung genutzt wird. Allerdings gewichten Fledermäuse Flussinformation geringer, als visuell orientierte Flieger, wahrscheinlich weil über das Echo Delay direkte Distanzinformation verfügbar ist.

Der zweite Teil der Dissertation befasst sich damit, wie Phyllostomiden ihre zum Echoortungssystem gehörenden Gesichtsstrukturen zur Analyse von echo-akustischem Fluss einsetzen. Dazu wurden Bewegungen des auffälligen Nasenblattes und der Außenohren, sowie das allgemeine Echoortungsverhalten beobachtet, während die Fledermaus (passiv) entlang unterschiedlich strukturierter Seitenwände bewegt wurde. Ich beobachtete Bewegungen sowohl des Nasenblattes, als auch der Außenohren, welche mit dem Aussenden von Echoortungsrufen korrelierten. Vor einem Echoortungsruf wurde das Nasenblatt angespannt und entspannte sich im Anschluss daran. Das Anspannen des Nasenblattes führte zu einem Beugen oder Strecken des Nasenblattes. Gemäß vorangegangener Studien und Simulationen scheint es wahrscheinlich, dass diese Nasenblattbewegungen dabei helfen die bei der Rufaussendung entstehende Schallkeule anzupassen, indem der Schall in der Vertikalen gelenkt wird. Dies erlaubt das Abtasten unterschiedlicher Regionen im Raum. Außerdem traten Ruf-korrelierte Ohrbewegungen auf, die einem stereotypen Muster entsprachen: beide Ohren wurden in eine aufrechte Position aufgestellt und nach vorne gerichtet, bevor die Ohren wieder abwärts in eine eher seitlich-hängende Position bewegt wurden. Ich schlage vor, dass Ohrbewegungen dazu dienen die Sensitivität des empfangenden Systems dynamisch an die Anforderungen der auditorischen Szene anzupassen. Deshalb wurde in einem weiteren Experiment getestet, ob der Zeitpunkt, zu dem die Ohren maximal aufgestellt (und vermutlich die Sensitivität des empfangenden Systems am höchsten) waren, mit der Distanz zu den Reflektoren korreliert. Zu diesem Zweck beobachtete ich Ohrbewegungen, während die Fledermaus auf flankierende Säulenreflektoren zubewegt wurde. Die meisten Fledermäuse stellten die Ohren später (relativ zur Rufaussendung) und mehr auf, wenn sie noch weit von den Reflektoren entfernt waren und stellten die Ohren früher (relativ zur Rufaussendung) und weniger auf, wenn die Distanz zu den Reflektoren kürzer war. Das lässt darauf schließen, dass Phyllostomiden das Timing und das Ausmaß der Ohrbewegung mit Millisekunden-genauer Präzision an die Distanz zu den Reflektoren anpassen können.

Abstract

Bats are nocturnal animals that navigate swiftly and skilfully in the dark using echolocation. They emit ultrasonic echolocation calls that are reflected by nearby objects in the surroundings. By analysing differences between the emitted echolocation call and the returning echo, bats can extract information about an object's shape, size, orientation, texture and three-dimensional location. During flight, the bat moves relative to its surroundings and accordingly experiences a flow of echo-acoustic information from the various, nearby objects.

This cumulative thesis comprises two independent studies on the perception of echo-acoustic flow in phyllostomid bats. The first part of the thesis addresses the question, if bats rely on echo-acoustic flow information for flight guidance. In animals that rely on visual information for navigation, optic flow is an important cue. It enables these animals to assess their relative position to the surroundings and it is used for the guidance of movements. In the first experiment I tested the hypothesis, that bat flight through narrow passages is affected by echo-acoustic flow. To this end, bats were trained to fly through a narrow corridor with structured side walls. Indeed, the bats adjusted flight paths according to the strength of echo-acoustic flow elicited by the side walls: the bats centred flight paths around midline, when both sidewalls produced echo-acoustic flow of equal strength, and shifted their flight paths towards the side producing weaker echo-acoustic flow, when the strength of echo-acoustic flow differed across the side walls. These flight path adjustments are analogous to those displayed by the visually guided flyers (i.e. birds and flying insects). This is a surprising finding, considering that echolocation, unlike vision, provides direct distance information via echo delay (i.e. the time elapsing between the emission of an echolocation call and the reception of the echo). However, the bats ascribed different perceptual weightings to side walls producing weaker or stronger echo-acoustic flow. Furthermore, I analysed if bats adjusted other aspects of their flight and echolocation behaviour to echo-acoustic flow. The animals did not adjust flight speed to echo-acoustic flow, an effect readily observed in visually guided flyers. Echo-acoustic flow had a weak effect on ensonification rates and temporal ensonification patterns, indicating that the bats only slightly adjust their echo-acoustic

sampling strategy to the strength of echo-acoustic flow. Overall, our results show, that echo-acoustic flow is used for flight guidance by echolocating bats. However, the bats perceptually rank flow information lower than visually guided flyers, probably due to the availability of direct distance information via echo delay.

The second part of the thesis addresses the question, how phyllostomid bats employ facial features of their echolocation system for the analysis of echo-acoustic flow. To this end, movements of the prominent noseleaf and the pinnae as well as the general echolocation behaviour were monitored, while the bat was (passively) moved along differently structured side walls. I found movements of both the noseleaf and the outer ears, that were correlated with the emission of echolocation calls. Before an echolocation call, the noseleaf tensed and relaxed thereafter. Tensing of the noseleaf caused the noseleaf to either bend or stretch. Based on previous studies and simulations, it seems likely that noseleaf movements aid adjusting the sonar beam by redirecting sound to different elevations to the front of the bat, allowing the scanning of different regions in space. Additionally, the bats produced call-correlated ear movements that conformed to a stereotyped pattern: both ears were raised and set in an upright position, pointing both ears towards the front, before the ears moved downward again to a more lateral and suspended position. I suggest that ear movements serve to adjust the sensitivity of the receiving system dynamically, according to the needs imposed by the auditory scene. Therefore, a further experiment investigated, if the time when the ears are maximally raised (and supposedly the directionality of the system is highest) correlates with the distance to the reflectors. To this end, I monitored ear movements, while the bat was moved towards flanking column stimuli. Most bats raised the ears later with respect to call emission, and to a higher extent, when the bats were still further away from the reflectors. They raised their ears earlier and to a lesser extent, when the distance to the reflectors was shorter. This suggests that phyllostomid bats can adjust the timing and the magnitude of these ear movements to the distance of the reflectors with millisecond precision.

Contents

1 General Introduction

Flow fields arise due to relative motion between an observer and nearby objects. Sensory flow depends both on the information received by the sensory systems and on the (self-) motion of the observer. In the following, I will first address how sensory systems acquire information about the environment, especially how spatial perception is achieved via sensory systems. Furthermore, I will elaborate on how motion and adaptive strategies can influence the perception of sensory flow.

1.1 Sensory systems determine the perception of the environment

Animals live in complex environments. These environments are composed of a vast set of biotic and abiotic factors with different characteristic, physical properties. Animals acquire information about their surrounding environment via their various sensory systems. There are different sensory systems responding to stimuli with different physical properties: Visual systems detect electromagnetic waves (light) in a certain range. The human visual system for example responds to electromagnetic signals with wavelengths between 400-700 nm, whereas signals with shorter or longer wavelengths do not elicit a response (Bear et al., 2007). Auditory systems detect pressure changes that propagate from a vibrating source through a transmission medium as mechanical waves (sound). The human auditory system detects sounds within the frequency range between 20 Hz and 20 kHz (Bear et al., 2007). Other mechanoreceptors respond to different mechanical stimuli or forces, like e.g. the proprioceptive system that monitors body posture and movements by detecting distortions and mechanical forces from muscles, joints and the skin. Olfaction (smell) and gustation (taste) detect airborne and other chemical signals (Bear et al., 2007).

The general structure of all sensory systems (e.g. the visual or the auditory system) consists of peripheral accessory structures (e.g. the cornea and the lens in the visual, or the outer and middle ear in the auditory system) followed by a modality-specific transduction mechanism. Signal transduction takes place in the sensory epithelium (e.g. the retina in the eye, or the organ of Corti in the ear) which contains receptor cells (e.g. photoreceptors of the retina, or hair cells within the organ of Corti) that respond to the physical stimulus. Sensory receptors transduce the physical stimuli into changes in receptor potential and ultimately in neural signals (action potentials). These neural signals travel through the corresponding sensory circuits up to the brain where they are further analysed and processed.

In order to get around and navigate through their surrounding environments, animals face the challenge to analyse only the behaviourally relevant stimuli and to obtain only the purposeful information (Wehner, 1987). While it might be physiologically possible to sense stimuli beyond the sensing range of the sensory system, this could in fact be disadvantageous for the animal. Too much information impedes the detection and classification of the relevant stimuli and its acquisition comes with additional energetic costs (Niven and Laughlin, 2008). This is why sensory systems evolved to be selective for certain physical stimuli while they do not detect others. This selectivity is called sensory filtering. Maximising the explanatory power by sensory filtering leads to the evolution of filters that are matched to the stimulus range that is ecologically relevant to the animal. These so-called matched filters simplify the extraction of only vital information (von der Emde and Warrant, 2015; Wehner, 1987; Warrant, 2016). Furthermore, they determine an animal's internal representation and perception of the environment (von der Emde and Warrant, 2015).

1.2 Passive and active sensory systems

Most sensory systems are passive systems. They pick up stimuli that arise upon the interaction of the environment with energy provided by an external source (c.f. Nelson and MacIver 2006). For instance, the auditory system detects sound produced by an external source, e.g. a radio. Another example is the visual system. Objects reflect light provided by an external source like the sun. The reflection can be detected and characterized by visual systems. The human visual system performs well during the day, as the electromagnetic energy (visible light) emitted by the sun is sufficiently high. At night, however, the energy provided by the moon and the stars might not suffice for good human vision. Due to these energetic limitations, some animals have evolved active senses: in active sensory systems, the sensory system itself produces the energy that is required to evoke the information-bearing stimulus a stimulus, which can then be received by the same sensory system. Examples for active senses are active whisking, active sniffing, active touch (haptics), bioluminescence-aided vision, active electrolocation and echolocation (Nelson and MacIver, 2006).

1.3 The active sense of echolocation

Echolocation is an active auditory sense. Echolocating animals emit sounds, the echolocation calls, which are reflected by nearby objects (see Fig 1.1A). The reflected echoes are then analysed and information about the objects' position and characteristics are extracted (reviewed in Neuweiler, 2000; Thomas, 2004). Echolocation systems with different levels of complexity have evolved independently in various animal species. All echolocators share a common challange: they navigate in conditions where visual information is not reliable, e.g. in murky water or darkness. Basic forms of echolocation were reported for cave-dwelling birds (*Aerodramus* swiftlets and oildbirds), shrews, tenrecs and rats (Gould, 1965; Gould et al., 1964; Griffin, 1953; Novick, 1959; Riley and Rosenzweig, 1957). Humans, especially blind subjects, can also be trained in echolocation and some subjects perform surprisingly well (reviewed in Kolarik et al., 2014). Elaborate echolocation, however, has only been identified in bats and toothed whales (Griffin and Galambos, 1941; Norris et al., 1961; reviewed in Thomas, 2004). The ability to echolocate gave both groups access to a new ecological niche. Echolocating whales can dive deeper into the ocean compared to their non-echolocating relatives, where light is too scarce for visual navigation (Nummela et al., 2004). In bats, echolocation in combination with their ability for active flight enables these nocturnal animals to navigate and hunt, even in absolute darkness.

1.3.1 3D object localization via echolocation

Research on echolocation in bats has revealed how these animals extract features of nearby objects. The term echolocation itself suggests that it allows for the localization of objects based on (echo-) acoustic information. Auditory and visual object localization are fundamentally different. In the visual system, the sensory epithelium is arranged along a space axis and directly encodes the azimuth (horizontal location) and elevation (vertical location) of objects. In the auditory system, however, the sensory epithelium is arranged along a frequency axis and the location of an object must be computed. Different mechanisms are involved in the localization of an object's azimuth, elevation and range (distance):

When an object is located further to the left of a bat, the echo will travel a shorter distance to reach the left, and a longer distance to reach the right ear. This causes the sound to arrive earlier at the left ear than at the right ear, giving rise to Interaural Time Differences (ITDs, see Fig 1.1C). Furthermore, sound is subject to attenuation, i.e. a gradual loss of intensity that scales with propagation distance. Therefore, the echo from an object to the left will not only arrive earlier, but also with a higher intensity at the left ear than at the right ear. These differences between the ears are so-called Interaural Level Differences (ILDs, see Fig 1.1C). ITDs and ILDs can be converted into the object's azimuth, i.e. its location in the horizontal dimension (Erulkar, 1972; Heffner et al., 2010; Neuweiler, 2000; Rayleigh, 1907; Wohlgemuth et al., 2016b).

To understand how it is even possible for the auditory system to compute elevation, one first needs to understand how sound travels to the ear and is modified in this process. When an echo is reflected from an object back to the bat, most of the sound will not directly enter the ear, but first hit different morphological features of the ears, the head and the torso. These structures reflect the sound, depending on the angle of incidence and the curvature of the reflective surface. The angle of incidence and the curvature of the reflective surface are not the only factors that determine how a sound is reflected. As previously described, sounds arise when a vibrating object induces density fluctuations in a transmission medium. When the density fluctuates with one constant speed, this will produce a fixed number of fluctuations per second and the arising sound has a distinct frequency. Sound frequency is inversely proportional to the signal's wavelength. The wavelength of a signal is another factor that determines how a sound is reflected from a surface. High-frequency sounds are reflected differently from the same morphological structure than low-frequency sounds. Biologically produced sounds are complex: they are composed of many different frequencies with different amplitudes, which can be described by the frequency spectrum of the signal. When a complex, biological sound, like an echo, is reflected towards the animal, various reflection patterns will arise for the different frequency components in the signal. These interact and interfere constructively and destructively. Consequently, the echo undergoes frequency-specific amplification or attenuation, respectively. As a result, the original frequency spectrum of a sound is modified when reaching the eardrum. The described interactions depend on the position of the reflective object. This phenomenon is specified by the head-related transfer function (HRTF). The HRTF characterizes the ratio of the frequency spectrum of the original sound (coming from a fixed point in space) and its modified version at the eardrum. In other words, the HRTF describes the (positive or negative) gain in sound intensity at the eardrum compared with the original frequency spectrum of the signal (Moore, 2012). The arising spectral cues (i.e. frequency cues) provide the basis for localization in elevation (see Fig 1.1D). To a lesser degree, they contribute to localization in azimuth (Aytekin et al., 2004; Neuweiler, 2000; Wohlgemuth et al., 2016b; Wotton et al., 1996).

Determining an object's range is not trivial for most sensory systems. For example in vision, object range needs to be computed by triangulating information from multiple perspectives, typically the perspectives from both eyes (binocular vision, producing binocular parallax) for stationary objects or the perspectives accumulated over time (producing motion parallax) for objects that move with respect to an observer (Gibson, 1950; Lee et al., 1992). The range of an object can also be estimated exploiting monocular cues like texture and density gradients (Gibson, 1950). Notably, active senses like echolocation have direct access to distance information: they can exploit the fact that time passing between the emission of energy and the reception of the reflection provides a distance estimate: sound propagates with constant speed (speed of sound) through air, when air pressure, temperature and humidity are constant. Consequently, the delay between the emission of an echolocation call and the reception of the echo (echo-delay, see Fig 1.1B) is a direct measure of the propagation distance and encodes for the target range (Neuweiler, 2000; Simmons, 1973).

1.3.2 Directional emission and reception of echolocation calls and echoes

The second important component of the echolocation system is the emitter, where echolocation calls are produced and emitted. Bats produce their echolocation calls and other vocal signals in the larynx. Air is expelled from the lungs and exhaled over the vocal folds. In response, these start to vibrate in different frequencies, depending on the shape and tension of the vocal folds. Their shape and tension are under muscular control. The vibration frequency of the vocal folds determines the frequency spectrum of the produced sound. After production, the sound is funnelled via the vocal tract to the emitter. During this process some frequencies components of the signal are amplified and others attenuated, due to constructive and destructive interferences within the vocal tract. Subsequently, the echolocation calls are emitted. Some bats, e.g. those belonging to the families of verspertillionid and emballonurid bats, emit echolocation calls through the open mouth (orally). Others, e.g. those belonging to the families of rhinolophid, hipposiderid and phyllostomid bats, emit echolocation calls through their nostrils (nasally). In orally emitting bat species, the modifications of the call's frequency spectrum that are introduced in the vocal tract are rather subtle, while they are

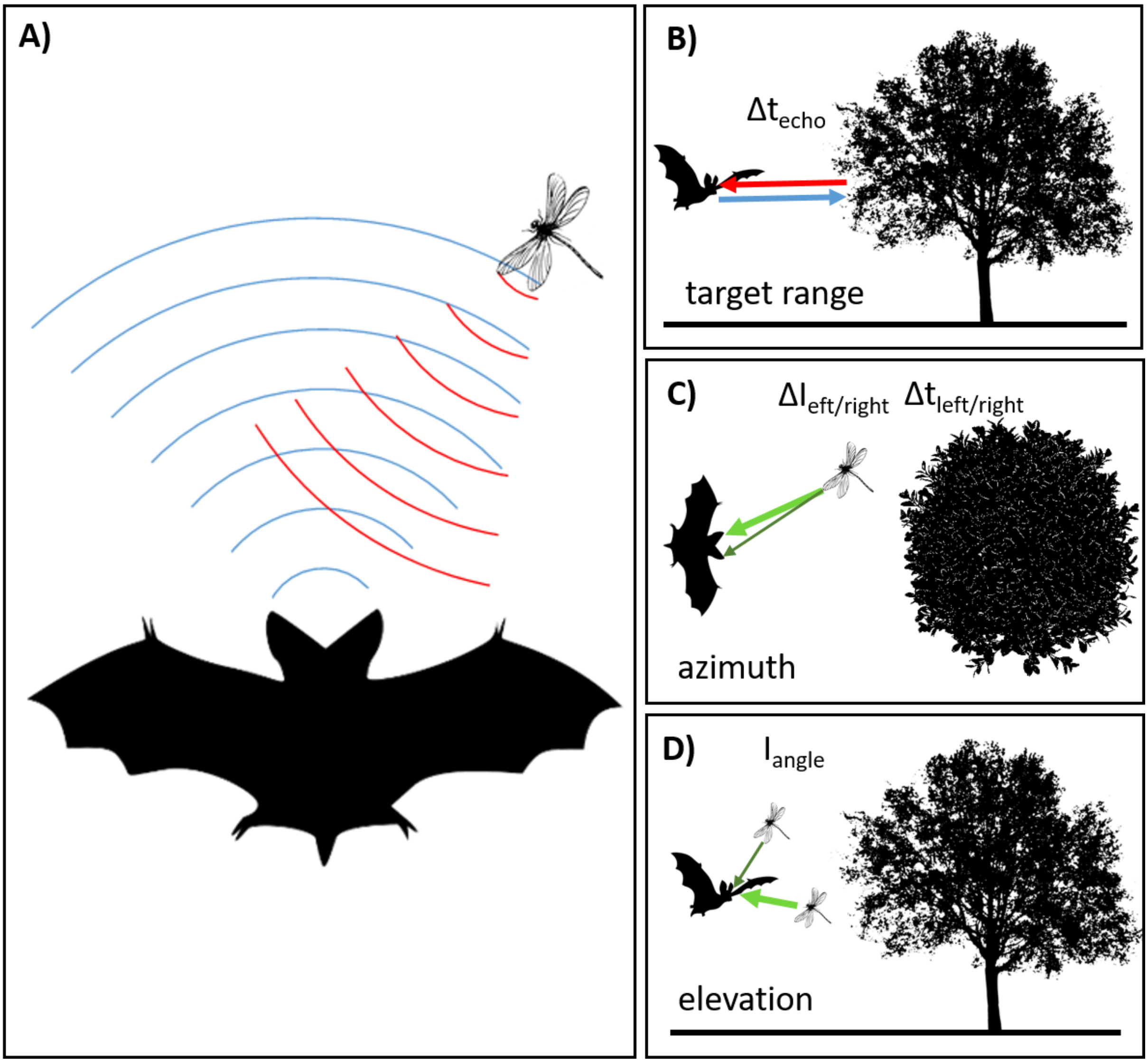

Figure 1.1: A) Echolocating bats emit echolocation calls that are reflected by nearby objects. From information encoded in the returning echoes, they can extract information about the object's position in space and also about its 3D shape. B)-D) show a bat flying and hunting insects in front of a tree. B) Side view of the scene. The distance to an object (target range) is determined by measuring echo delay C) Top view of the scene. Differences between the ears in intensity (interaural level differences, ILDs) and arrival time (interaural time differences, ITDs) provide the main cues for localization in the horizontal dimension (azimuth). D) Side view of the scene. Echoes from objects at different positions in the vertical dimension (elevation) arrive with an angle- and frequency-dependent intensity at the ear drum. The bats use these spectral cues for localization in elevation.

more prominent in nasally emitting species (Neuweiler, 2000).

Upon emission, the echolocation beam is directional: most of the sound energy is directed towards the front of the bat and falls off towards the sides. The aperture of the emitter determines the degree to which the energy is focussed towards the front. Orally emitting species can therefore easily alter the directionality of the emitted call: Kounitsky et al. (2015) experimentally proved the suggestion by Surlykke et al. (2009a) that bats achieve an increased directionality by opening their mouth more, and a decreased directionality by opening their mouth less. Such directionality of the emitted call can also be introduced by morphological traits: when sound is emitted not from one (open mouth), but from two point sources (nostrils), interference between the two emitted, spherically spreading waves cause frequency-dependent amplification and attenuation and can therefore alter the directionality of the echolocation beam (Hartley and Suthers, 1987; Neuweiler, 2000). It was found that the resulting interference patterns depend predominantly on the distance between the nostrils. This factor affects mostly the directionality in azimuth, i.e. the directionality along the horizontal dimension (Hartley and Suthers, 1987). The noses from nasally emitting bats often carry conspicuous structures called noseleaves. These motile, fleshy appendages influence the frequency content, i.e. the spectral characteristics, of the emitted call. Simulations and modification experiments revealed that noseleaves influence the directionality of the echolocation beam in elevation (Hartley and Suthers, 1987; Vanderelst et al., 2010). Furthermore, their size also determines the aperture of the emitter and thus influences the directionality of the emitted call.

Finally, bats exploit the fact that high frequency sounds show different propterties than low frequency sounds. The emitted sound energy undergoes attenuation, or spreading losses, upon propagation, which partly depend on sound frequency. Geometric attenuation denominates the reduction in sound intensity with distance, as sound energy is spread across a larger surface. This process affects all frequencies equally. In addition to geometric attenuation, sound energy is absorbed when passing through air. This so-called atmospheric attenuation depends on air humidity, temperature and pressure as well as on sound frequency. It effectuates that higher frequencies fall off steeper than lower frequencies and is thought to dramatically limit the range of echolocation (Griffin, 1971; Lawrence and Simmons, 1982). However, to increase the directionality of their echolocation calls, bats typically employ echolocation calls in the ultrasonic range, i.e. echlocation calls with frequencies above 20 kHz that exceed the human hearing range. The facts that (1) bats can achieve higher spatial resolution via the use of high-frequency signals and that (2) the echolocation calls may be above the hearing range of most prey and predators constitute advantages of high-frequency signals (Neuweiler, 2000).

Not only the emitting, but also the receiving system of bat echolocation is directional. The receiving system comprises the outer ears, or *pinnae*, and the ear canal. It connects to the eardrum. As mentioned before (see section 1.3.1 and Fig 1.1D), the pinnae and the anatomy of the entire head act as a filter that reinforces and attenuates signals in a direction- and frequency-dependent manner as described by the HRTF. The gain pattern is more focused for higher frequencies (De Mey et al., 2008; Obrist et al., 1993) because the outer ears are large with respect to the ultrasonic signal's wavelength (Neuweiler, 2000). Gain is usually high for signals originating from more central, and lower for signals originating from more peripheral locations. This means that there is a main axis of the ear directionality. From studies in cats it is known that with a change in ear posture, the main axis of the directionality changes accordingly (Young et al., 1996). This effect is also apparent in bats. Additionally, it was found that an upright posture of the bats' ears results in a rather forward oriented directionality (Gao et al., 2011; Vanderelst et al., 2010).

The directionality of the emitting and the receiving system of bat echolocation causes the volume in space being sampled with one echolocation call (the sonar detection range) to be quite restricted (Ghose and Moss, 2006; Jakobsen et al., 2013; Madsen and Surlykke, 2013). Therefore, despite constituting a remote sense, echolocation has a much smaller sensing range than e.g. vision. In active senses, a restricted sensing volume is quite typical, due to several reasons. First, directionality can be an advantage: it allows focussing on information from a defined region in space, while reducing information originating from regions of secondary importance (clutter). Secondly, the signal intensity in the direction of sound emission is higher: upon propagation, the emitted energy undergoes attenuation, also termed spreading losses. For active senses, the propagation distance is twice as long as for passive senses, because the energy has to propagate towards an object and back. Therefore, the spreading losses are even higher than for passive senses (Jakobsen et al., 2013; Nelson and MacIver, 2006). Directing most of the energy towards the front in order to achieve a larger sensing range at the expense of lateral sensing volume is one way to counteract such spreading loss (Jakobsen et al., 2013). Another technique to increase echolocation range is calling at lower frequencies. As stated above, attenuation in air is more severe for high than for low frequencies. Therefore, loud echolocation calls with a low peak frequency are well suited for bats foraging in open space. By this means the detection range is increased (Neuweiler, 2000).

1.4 Task-specific adaptations in echolocation behaviour

Indeed, bats can voluntarily change call and beam characteristics. This allows them to adjust their echolocation calls flexibly to a given situation: *Marophyllum macrophyllum*, a phyllostomid bat species, emits echolocation calls at varying intensities, depending on the degree of clutter in the environment. Brinkløv et al. (2010) discovered that *Marophyllum marcrophyllum* emits fainter calls in a small flight room (i.e. in cluttered space), increases call level in a semi-cluttered environment and calls even louder in open space. Another example of voluntary changes in call characteristics are the well documented range-dependent adjustments of different call parameters: many bats increase the bandwidth of their echolocation calls and reduce call intensity, inter call interval and call duration, when approaching a target (reviewed in Hofmann et al. 2013; Moss and Surlykke 2010). Linnenschmidt and Wiegrebe (2016) showed that *Phyllostomus discolor*, another phyllostomid bat species, can dynamically adjust its sonar beam shape when tracking an approaching target. Apart from beam shape, bats can also change beam orientation: An experimental approach in the lab revealed that bats of the species *Eptesicus fuscus* which were trained to fly through holes in a fine net in order to get a food reward, scan the edges of the holes before passage (Surlykke et al., 2009b). Another study by Seibert et al. (2013) found that under natural conditions pipistrelle bats can orient echolocation calls in a rather wide cone surrounding the heading direction. The bats often change beam orientation from one call to the next. A further example how animals actively adapt echolocation behaviour is prey interception. In most bat and odontocete species that hunt moving prey, foraging behaviour follows a rather stereotyped pattern (Griffin, 1958; Madsen and Surlykke, 2013; Miller et al., 2004) comprising the following three phases (Geberl et al., 2015; Griffin, 1958; Simmons et al., 1979): during search flight (1), bats emit echolocation calls at a rather low repetition rate. When detecting prey, the bat starts the approach phase (2) and call repetition rate increases dramatically while call duration decreases. Finally, in the terminal buzz phase (3), just before prey capture, calls are repeated at very high rates of 180 times per second or more, and the bats decrease the peak frequency of their echolocation calls. In echolocation, information is not acquired constantly, but only when the animal actively emits an echolocation call. Therefore, the successive increase in call repetition rate allows for faster updates. Shortening the call duration ensures that, even at the higher call repetition rates, there is no overlap, and thus no ambiguity, between echoes to different calls (reviewed in Moss and Surlykke 2010). During the buzz, the bats also adjust call intensity and the sensitivity of the hearing system (Suga and Jen, 1975), a process referred to as automatic gain control (Hartley, 1992a,b; Hiryu et al., 2007). Due to these adjustments, the returning echoes are relatively stable in their perceived intensity (Hartley, 1992a; Henson, 1965; Suga and Jen, 1975).

These are only few out of many examples of how a bat can actively influence, and by implication optimize, the shape and load of sensory information to be processed.

1.5 The importance of (self-) motion for perception and navigation

As mentioned in the beginning of this introduction, sensory flow itself is the change of sensory information over time. It arises when an animal moves relative to an object it senses (see Fig 1.2), because (self-) motion induces correlated changes in the percept of the environment (Gibson, 1950; Lee et al., 1992). For example, when an animal moves along a path with a laterally placed object, the angle between the object and the motion path gradually expands while the distance between the animal and the object diminishes until the animal passes the object. Naturally, the perception of self-motion is crucially important for navigation. It allows to determine the time to contact with an object, the animal's heading direction and velocity as well as depth information (Cornilleau-Peres and Gielen, 1996; Gibson, 1950, 1979; Lee, 1976). For flying animals, sensory flow is hence important to accomplish various tasks like landing, avoiding obstacles or interception of prey. Due to the close link and the mutual interaction between action and perception, active shaping of sensory flow can be achieved, if either the animal itself or a sensor is moved relative to the stimulus. Hofmann et al. (2013) coined the term *active sensing strategies* for the active shaping of sensory flow that can follow two objectives: (1) the selection or shaping of sensory streams that impinge on the sensors and (2) the generation of spatio-temporal dynamics in the sensory flow that aid in the extraction of information. Adaptive strategies can serve either one or both of these objectives. For instance, a directed orientation of sensors with respect to the stimuli of interest can help to focus on some sensory streams and to ignore others. Another example would be the precisely timed emission of echolocation calls that helps adjusting the spatial and temporal resolution to the needs imposed by the current task.

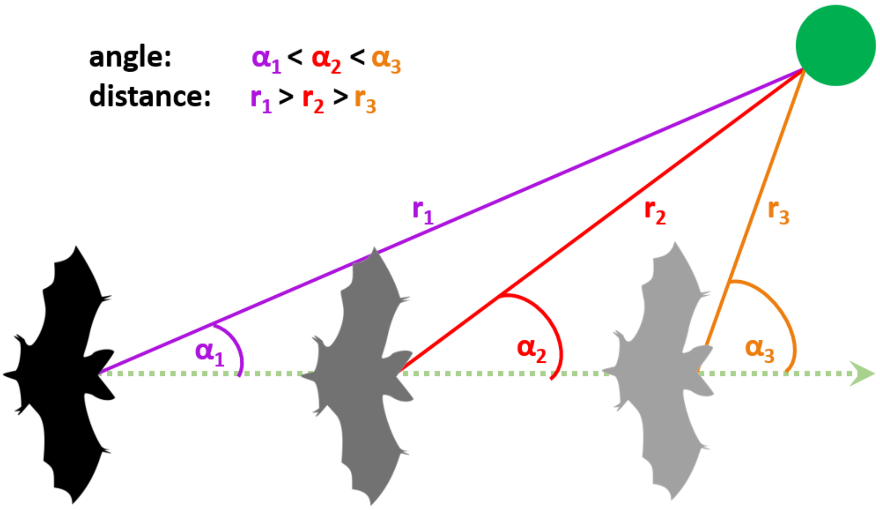

Figure 1.2: Sensory Flow. As an echolocating bat passes an object (green circle), the geometric relation between the bat and the animal changes in time. In this example, the angle between flight direction and object increases, while the distance between the bat and the object decreases. Consequently, the sensory information changes systematically and produces sensory flow.

Both types of active sensing strategies shape sensory flow, and consequently shape the information sensed by an animal. This information determines the internal representation of the environment and hence, how the animal perceives its environment.

1.6 Experimental aim

This thesis comprises two behavioural experiments addressing the question, whether phyllostomid bats (*Phyllostomus discolor*) apply active sensing strategies that involve the shaping of echo-acoustic, sensory flow.

Chapter 2 of this thesis addresses how the perception of echo-acoustic flow influences flight behaviour. We investigated how flying bats align their body, and thus voluntarily alter perceived echo-acoustic flow, in response to different echo-acoustic flow fields. Not only body alignment, but also the sampling strategy determines the perceived echo-acoustic flow. Therefore, this chapter also focuses on the accompanying changes in echolocation behaviour.

In Chapter 3 of this thesis, we address how *Phyllostomus discolor* samples and analyses echo-acoustic flow. Specifically, we ask how this bat species deploys the facial features of its echolocation system for a comprehensive echo-acoustic exploration of its surroundings during motion. This comprises active sensing strategies addressing call emission, including the nature and prospective function of noseleaf movements for call emission; it further involves an active sensing strategy addressing call reception, including the alignment of the outer ears, while echolocating during motion. We investigated how *Phyllostomus discolor* employs movements of the facial structures of its echolocation system to echo-acoustically investigate its surroundings while being moved along symmetrically flanking, structured surfaces creating different flow fields. In a second set of experiments, we tested if the bats adaptively adjust their typical, call-correlated ear movements in a range-dependent manner when approaching potential obstacles.

2 Echo-acoustic flow affects flight in bats

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SHORT COMMUNICATION

Echo-acoustic flow affects flight in bats

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ABSTRACT

Flying animals need to react fast to rapid changes in their environment. Visually guided animals use optic flow, generated by their movement through structured environments. Nocturnal bats cannot make use of optic flow, but rely mostly on echolocation. Here, we show that bats exploit echo-acoustic flow to negotiate flight through narrow passages. Specifically, bats' flight between lateral structures is significantly affected by the echo-acoustic salience of those structures, independent of their physical distance. This is true even though echolocation, unlike vision, provides explicit distance cues. Moreover, the bats reduced the echolocation sound levels in stronger flow, probably to compensate for the increased summary target strength of the lateral reflectors. However, bats did not reduce flight velocity under stronger echo-acoustic flow. Our results demonstrate that sensory flow is a ubiquitous principle for flight guidance, independent of the fundamentally different peripheral representation of flow across the senses of vision and echolocation.

KEY WORDS: Echolocation, Sonar, Navigation, Flow field, Flight guidance

INTRODUCTION

Flight allows for fast navigation in 3D space. For visually guided animals in flight, stationary objects in the close environment produce patterns of visual motion on the retina, commonly referred to as optic flow (Gibson, 1994). Numerous studies have shown that optic flow is an important entity in perception that guides motion in space in many flying animals (Bhagavatula et al., 2011; Dyhr and Higgins, 2010; Frye and Dickinson, 2007; Srinivasan, 1996). Optic flow is also important for avoiding collisions or estimating time to contact (Wagner, 1982; Wang and Frost, 1992).

Bats, as the only flying mammals, typically cannot use vision to negotiate flight close to structures because of their nocturnal life style. Instead, bats mostly rely on echolocation, i.e. the auditory analysis of the echoes of self-generated sounds that are emitted at varying rates (Griffin and Grinnell, 1958). Unlike vision, echolocation provides explicit distance information through the analysis of echo delay (Simmons, 1971, 1973). Specifically, at a given speed of sound, the delay between a sonar emission and the reception of the echo encodes the distance of the reflective surface in metres. Bats have evolved specialised neural circuits in the auditory brainstem and midbrain to measure echo delay, resulting in a cortical chronotopic map (O'Neill and Suga, 1979; Portfors and Wenstrup, 1999, 2001; Suga, 1970, 1990). Azimuth and elevation of a reflective surface have to be binaurally computed, because the sensory epithelium for sound (the organ of Corti) does not provide explicit spatial information. Thus, compared with vision, echolocation provides relatively sparse spatial information in azimuth and elevation, but more explicit distance information.

For a bat flying through structured 3D space, distances and angles of sound-reflecting surfaces change continuously, effectively creating an echo-acoustic flow. This continuous flow, however, is discretely sampled by bats and thus is not necessarily perceived as continuous.

Echo-acoustic flow is especially required during commuting flight – when bats follow the edges of vegetation to travel between roosting and feeding sites – in order to keep the lateral distance to passing objects constant, as is typically observed in field studies (Holderied et al., 2006).

Both experimental and theoretical work has indicated that bats might perceive echo-acoustic flow (Bartenstein et al., 2014; Lee et al., 1992; Müller and Schnitzler, 1999, 2000) but the unequivocal use of echo-acoustic flow for navigation by bats has not been demonstrated. Here, we tested the hypothesis that, despite the explicit distance information provided by echolocation, bats still recruit echo-acoustic flow field information to adjust their distance from lateral structures in flight. If this were true, we would expect bats flying between structures that differ in the strength of their echo-acoustic flow to fly closer to the structure that elicits the weaker flow.

MATERIALS AND METHODS

Experimental setup

The experimental setup was a flight tunnel (Fig. 1A) consisting of two echo-attenuated, terminal cubes and a 3 m-long, removable test zone in between. The terminal cubes could be separated from the test zone with curtains and each contained one feeder. Audio was recorded via four ultrasonic microphones (SPU0410LR5H, Knowles, Itasca, IL, USA) positioned centrally on the back walls of the terminal cubes (20 and 91 cm height). Analog signals were preamplified (Octopre LE, Focusrite, High Wycombe, UK) and AD converted by an audio interface (Ultralite, MOTU, Cambridge, MA, USA) at 192 kHz.

The floor of the test zone was lined with white cloth to achieve high contrast in the video; its ceiling consisted of visually and acoustically transparent gauze. It was lit along both side walls with infrared LED strips (Synergy 21 LED Flex Strip infrared 86417, ALLNET GmbH Computersysteme, Germering, Germany). An infrared camera (A602f, Basler, Ahrensburg, Germany) at 3.54 m height recorded videos at 20 frames s^{-1}. For audio-visual synchronization, the camera was triggered via the audio interface.

Stimuli and experimental procedure

Stimuli for this experiment were the structured side walls of the test zone. Each side wall was planked vertically on one side and horizontally on the other side with tongue-and-groove panelling, which creates periodic ridges and grooves. Vertical ridges induce

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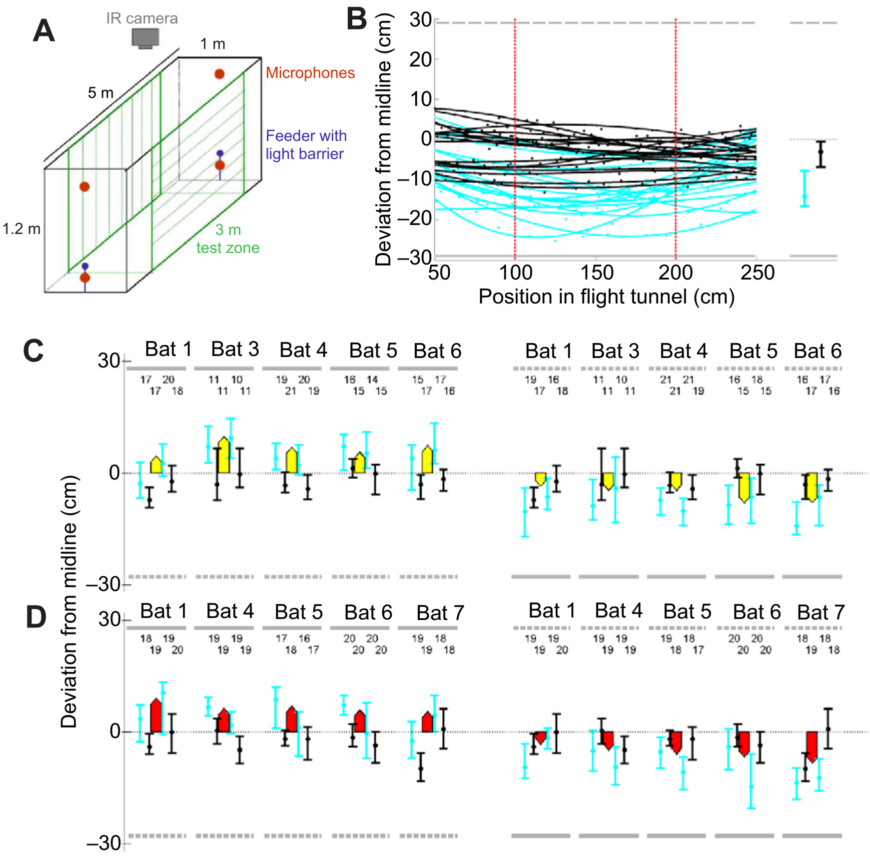

Fig. 1. Experimental setup and bat flight path analysis. (A) Schematic drawing of the set-up with a central test zone and two terminal cubes, separable with curtains. Bats' flights were monitored via an infrared (IR) camera centred above the test zone, and echolocation calls were recorded with two ultrasonic microphones (at 20 and 91 cm height at midline) in each of the terminal cubes. After each flight, the bat was rewarded from the feeder platform. The structures of the lateral walls in the test zone (either vertical or horizontal ridges) could easily be changed in between trials by rotating the lateral walls. (B) Left: example flight paths from bat 6 for lateral walls with orthogonal orientation to each other (blue paths, ridging illustrated by grey lines) and for lateral walls with concordant orientation (black paths, both walls with vertical ridges). Medians and interquartile range of the flight paths are shown on the right. (C) Medians and interquartile ranges of flight paths for all bats with broad ridges oriented either concordantly vertical (black) or orthogonally (blue). The panel on the left shows results for horizontal ridges on the 'upper' wall and vertical ridges on the 'lower' wall; the right panel, vice versa. The first two data points for each bat represent flights from left to right; the second two data points represent flights from right to left. The number of flights is given above the respective bar. Mean deviations between concordant and orthogonal ridges are represented by the coloured arrows. The direction and magnitude of the coloured arrows clearly show that the bats always flew closer to the side with the weaker echo-acoustic flow. (D) Data for the experiment with the fine ridges in the same format as C.

strong echo-acoustic flow because they are orientated perpendicular to the flight direction and result in time-variant echoes. Horizontal ridges induce weaker echo-acoustic flow because they are orientated parallel to the flight direction. Two experiments were conducted, using stimuli that vary in the strength of echo-acoustic flow when presented in vertical orientation: (i) broad panelling (weaker echo-acoustic flow) – ridge width 7.5 cm, groove width 1.5 cm; and (ii) fine panelling (stronger echo-acoustic flow) – ridge width 3 cm, groove width 1.5 cm.

Rotating the side walls changes the orientation of the ridges between horizontal and vertical. This resulted in eight experimental conditions [4 arrangements of walls (both vertical, both horizontal, one vertical and the other horizontal or vice versa) times 2 flight directions]. A sequence of random permutations of the eight conditions was pre-generated and experimental trials for each animal were acquired according to this sequence.

Training

Seven adult bats (3 female, 4 male) of the species *Phyllostomus discolor* Wagner 1843 were trained to fly back and forth between the two feeders. Five training days were followed by two resting days. Training took place in a dark, echo-attenuated flight room (2.1×1.2×2.4 m).

Data acquisition

Data acquisition took place on 10 consecutive days. The start of a trial was initiated by opening the curtain to the test zone; after the animal had passed the test zone, the experimenter closed the curtain behind it. Audio and video ringbuffer recordings (5 s duration) were saved. All technical equipment was controlled with a custom-written Matlab program (MathWorks, Natick, MA, USA) using Soundmexpro (HörTech, Oldenburg, Germany), and the Matlab image acquisition and data acquisition toolboxes.

Data analysis

Only trials without reversal of flight direction were analysed. Custom-written Matlab programs were used for all data analyses: automatic 2D flight path reconstruction, calculation of flight velocity and audio analyses. Some trials could not be analysed for audio because of a microphone defect.

All analyses were applied on the individual level for each experimental animal. Tests comparing the broad and fine panelling were applied on the results of one specific bat, i.e. only on data recorded with bats which participated in both experiments.

Flight path analyses were performed using all data points in the respective combination of flight direction and wall arrangement that lay within the central 1 m of the test zone.

Homogeneity of flight paths

A Brown and Forsythe test was used to test whether an animal's flight paths in the central 1 m of the test zone showed equal variances with concordant vertical and concordant horizontal ridges. The range between the first and the third quartile was used as a measure for the homogeneity of the flight paths of all flights of one bat. These values were compared to determine with which ridge orientation flight paths were more homogeneous.

Analysis of number of calls and inter-call intervals (ICIs)

We tested whether the number of calls produced in the central 2 m of the flight tunnel differed for flights between vertically ridged walls and flights between horizontally ridged walls (two-sided Wilcoxon rank sum test). We checked with a Kruskal–Wallis test whether the distribution of ICIs differed across these conditions. The skewness of the distributions, which quantifies the extent to which a distribution deviates from symmetry, was calculated. A Gaussian distribution has a skewness of zero (fully symmetric) whereas the ICI distributions show an asymmetry in favour of short ICIs. In this case, the skewness is larger than zero.

Experiments were approved by the Regierung von Oberbayern (55.2-1-54-2532-221-14) and conducted under the principles of laboratory animal care and the regulations of the German Law on Animal Protection. Approval to keep and breed the bats was issued by Munich district veterinary office.

RESULTS AND DISCUSSION

Example flight paths of a bat from left to right between vertical, concordant ridges are shown in Fig. 1B (black paths). When ridging was changed to horizontal on one wall, the bat chose to fly significantly closer to that wall and, consequently, further from the vertically ridged wall (Fig. 1B, blue paths). The medians and interquartile ranges of the bat's deviation from the midline within the central 1 m of the corridor are shown on the right of Fig. 1B. Overall, 645 flight paths from 5 bats were analysed with a ridge spacing of 9 cm. Fig. 1C shows how median flight paths differed between orthogonal (blue) and concordant vertical (black) ridge conditions when the vertical ridges were presented on one wall (Fig. 1C, left) or the other (Fig. 1C, right). Net lateral deviations for both flight directions are represented by the yellow arrows. All bats consistently and significantly shifted their flight paths towards the wall with horizontal ridges inducing lower echo-acoustic flow ($P<0.05$, two-sided rank sum test).

We repeated the whole experiment, replacing the side walls with walls where the ridge spacing was reduced from 9 cm to 4.5 cm; 750 trials from 5 bats were analysed. Data show that with this finer spacing of the ridges, the bats also significantly deviated in their flight trajectory between concordant and orthogonal ridges, again flying significantly closer to the horizontal ridges (Fig. 1D, red arrows). The magnitude of this deviation, however, was not significantly different from that with the 9 cm spacing (Wilcoxon signed rank test).

Next, we compared peak flight velocity to assess whether bats adjust their flight velocity to balance echo-acoustic flow. With the 9 cm ridges, all bats tended to fly faster when the horizontal ridges were presented on both walls than with vertical ridges (Fig. 2A). This was, however, only significant for bat 5 ($P\leq0.05$, two-sided Wilcoxon rank sum test). With the finer spacing between ridges (Fig. 2B), the differences in flight velocity between concordant vertical and concordant horizontal ridges were even less pronounced. We conclude that flight velocity was not significantly affected by echo-acoustic flow as presented here.

We then tested how homogeneous the flight paths were between the lateral walls when both of them had either horizontal or vertical ridges. Specifically, we tested whether the stronger echo-acoustic flow of the vertical ridges leads the bats to fly along more homogeneous paths than with horizontal ridges. With broad ridge spacing, we found this to apply in 8 of 10 cases (5 bats times 2 flight directions). With fine ridge spacing, we found this to apply in 6 of 10 cases ($P\leq0.05$, Brown and Forsythe test). Note, however, that we also found the opposite effect (less homogeneous flight paths with vertical ridges) in 1 of 10 cases each for broad and fine ridges. Overall, the bats showed more homogeneous flight paths when exposed to stronger echo-acoustic flow.

The ultrasonic recordings during flights show that all animals produced significantly fainter calls when both walls were vertically ridged than when they were horizontally ridged ($P\leq0.05$, two-sided Wilcoxon rank sum test). This is true for both the broad ridge spacing (4715 calls from 595 flights; Fig. 3A) and the fine ridge spacing (5702 calls from 704 flights; Fig. 3B). Only call onsets (root mean square of the first 0.4 ms) were analysed to make sure that echoes from the reverberant test zone did not contaminate the analysis. The bats did not alter their rate of sonar emission, either per distance or per time, between the vertical and horizontal ridges. Again, this is true for both the broad and the fine ridge spacing (data not shown). However, some of the bats changed their temporal ensonification strategy: specifically, the distribution of ICIs changed significantly (see Fig. 3C–F for example ICI histograms of bat 1) in that the skewness of the ICI histogram became less positive when the vertical ridges were replaced with horizontal ridges. This means that, while the bats did not produce significantly more calls with vertical ridges, the ICIs were more often shorter. Quantitative results for the analysis on the skewness of ICI histograms are shown in Fig. 3G,H.

Taken together, the current psychophysical experiments show that echolocating bats (*P. discolor*) adjust their flight paths between structured surfaces according to the strength of echo-acoustic flow elicited by these surfaces. Our bats always chose to fly closer to the side wall that elicited the weaker echo-acoustic flow. This is surprising because, unlike all visually guided flyers (insects and birds), bats have explicit information about their distance to objects through the neural analysis of echo delay. Our data show that the perceptual valence of echo-acoustic flow was ranked over these explicit echo-acoustic distance cues. Thus, our results demonstrate that sensory flow elicited by self-motion is a ubiquitous principle for guidance of flight in the animal kingdom, independent of the sensory modality and the fundamentally different peripheral sensory representation of the perceptual cues mediating the flow information.

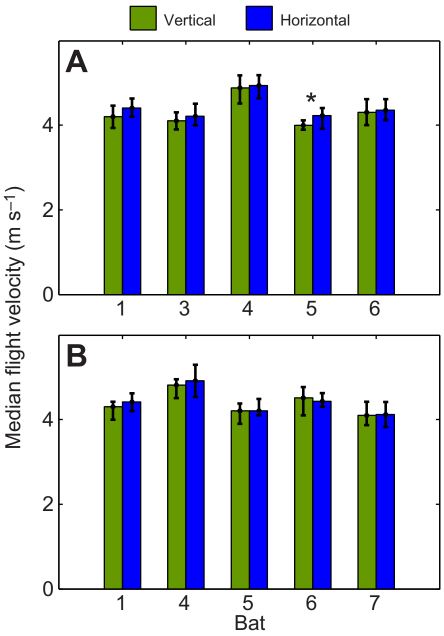

Fig. 2. Peak flight velocity. (A) Broad ridge spacing (9 cm). (B) Fine ridge spacing (4.5 cm). Green bars represent flight velocities with concordant vertical ridges; blue bars represent flight velocities with concordant horizontal ridges. Data are medians and interquartile range. The asterisk indicates a significant difference in flight velocity (Wilcoxon rank sum test, $P<0.05$).

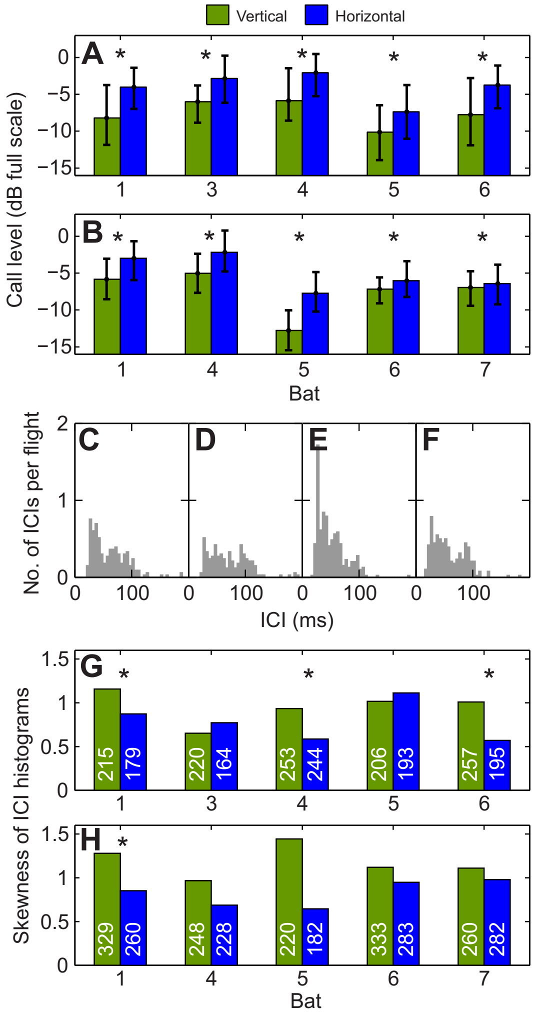

Fig. 3. Analysis of ensonification parameters. (A,B) Analyses of echolocation call levels for the broad (A) and fine (B) ridges. Data show that bats called significantly fainter with concordant vertical ridges (green) than with concordant horizontal ridges (blue). Data are medians and interquartile range. Asterisks show significant differences (two-sided Wilcoxon rank sum test, $P<0.05$). (C–F) Example inter-call interval (ICI) histograms of bat 1 (C, vertical broad ridges; D, horizontal broad ridges; E, vertical fine ridges; F, horizontal fine ridges). The ICI axis is truncated at 200 ms; maximal ICIs across bats and experimental conditions ranged between 102 and 233 ms. (G,H) Analyses of the skewness of the ICI distributions for the broad (G) and fine (H) ridges. Asterisks show where the distribution of ICIs differs significantly between concordant vertical ridges and concordant horizontal ridges. Numbers within the bars represent the number of ICIs on which each skewness analysis was based.

The neurobiological basis for the stronger perceptual valence of echo-acoustic flow compared with explicit echo-acoustic distance cues is not completely clear, but recent research has thrown some light on this. It has been known for some decades that target distance is explicitly encoded by specialised neurons in the brain of various bat species (Mittmann and Wenstrup, 1995; O'Neill and Suga, 1979; Olsen and Suga, 1991) and represented in a chronotopic map of echo delay in the bat auditory cortex (e.g. Bartenstein et al., 2014; Hagemann et al., 2010; O'Neill and Suga, 1979). However, it has recently been shown that this map is 'blurry' and responses depend critically on the actual combination of echo intensity and delay (Hechavarría et al., 2013). Indeed, Bartenstein et al. (2014) found that neurons in the auditory cortex encode echo-acoustic flow information on the geometric relationship between a target and the bat's flight trajectory, rather than echo delay. Thus, the classical chronotopic map as it has been described in the bat cortex may not encode echo delay per se. It may rather encode echo delay as it changes over time in typical fly-by situations that elicit echo-acoustic flow. This may provide a neurophysiological basis for the bats' perceptual preference described in our experiments.

How is echo-acoustic flow represented in the bat auditory system? Clearly the peripheral sensory representation of flow fields is fundamentally different across vision and echolocation: while in vision, the retina provides explicit spatial information for the time-variant structures, it is not even clear to what extent these structures are perceptually resolved in echolocation. A vertical ridge that generates the flow information in the current experiments can be approximated as a vertical line reflector that reflects into all azimuths. In response to a bat's call, many of these line reflectors will generate reflections that add up to a complex echo with increasing delays and decreasing amplitudes (due to geometric and atmospheric attenuation). Horizontal ridges, in contrast, will reflect relatively little energy back to the bat. Thus, it is conceivable that the bats did not perceive the vertical passing ridges as time-variant but simply as louder. In line with this, the bats reduced the call level with vertical ridges compared with horizontal ridges, an exemplification of automatic gain control, as ubiquitously observed in echolocating bats and whales (Au and Benoit-Bird, 2003; Hartley, 1992; Kick and Simmons, 1984; Linnenschmidt et al., 2012). However, the complex echoes generated by the ridges change periodically with a repetition rate equal to the product of ridge spacing and flight velocity. With the current (broad) ridge spacing of 11 m^{-1} and peak flight velocity around 4.5 m s^{-1} (see Fig. 2), echoes change periodically with a frequency of 49.5 Hz. But, our bats produced much fewer calls in flight, of the order of 16 s^{-1} (not shown). Thus, the ridge periodicity is strongly undersampled by the bats. Fontaine and Peremans (2011) have shown how bats can reconstruct the wing beat of insects despite such echo-acoustic undersampling, namely by adaptively adjusting and distributing ICIs. The fact that some of our bats indeed showed changes in the skewness of the ICI histograms (see Fig. 3C–H) corroborates this hypothesis.

In summary, the current experiments demonstrate that echolocating bats recruit flow field information to adjust their flight paths along structured layouts. These findings are in agreement with previous reports on birds and insects and thus corroborate the ubiquitous nature of flow field-guided navigation. However, bats assess lateral structures by echolocation, not vision, and the peripheral sensory representations of spatial information across these senses are fundamentally different. It is tempting to speculate that this difference results in the remarkable finding that our bats did not reduce flight velocity under stronger flow, an effect readily observed in birds and insects (e.g. Baird et al., 2005; Bhagavatula et al., 2011; David, 1982; Srinivasan, 1996). Our bats also did not change the ensonification rate and showed only small variations in temporal ensonification patterns. These data suggest that the explicit distance cues provided by echolocation, unlike vision, lead to a lower perceptual weighting of flow field information in bats compared with visually guided flyers. As all of our experiments were conducted in the dark, it may be informative in future studies to assess the extent to which bats flying in the light can recruit (optic) flow field information to negotiate flight through narrow passages. The relative salience of optic versus echo-acoustic flow field precepts may also vary strongly across bat species, as some bats are quite active in daylight or dusk (e.g. *Saccopteryx bilineata*), while many purely nocturnal, insectivorous bats appear to have somewhat reduced vision (e.g. *Pteronotus parnellii*).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
K.K., U.F. and L.W. designed the experiments; K.K. and W.G. built the setup; K.K. executed the experiments and analysed the data; K.K., W.G., H.L., U.F. and L.W. wrote the paper.

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3 Echo-acoustic scanning with noseleaf and ears in phyllostomid bats

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RESEARCH ARTICLE

Echo-acoustic scanning with noseleaf and ears in phyllostomid bats

Kathrin Kugler and Lutz Wiegrebe*

ABSTRACT

The mammalian visual system is highly directional and mammals typically employ rapid eye movements to scan their environment. Both sound emission and hearing in echolocating bats are directional but not much is known about how bats use ear movements and possibly movements of the sound-emitting structures to scan space. Here, we investigated in a tightly controlled behavioural experiment how *Phyllostomus discolor* bats employ their echolocation system while being moved through differently structured environments: we monitored and reconstructed both a close-up of the facial structures in 3D, including the motile noseleaf and outer ears, and the sonar-beam of the bat while it was moved along reflectors. Despite the simple linear movement of the bats in the setup, the bats pointed their beam quite variably in azimuth with a standard deviation of about ±20 deg. This variation arises from yaw-type head rotations. Video analyses show that the bat's noseleaf twitches with every echolocation call. Second, we show that the bat's ears are raised to a rather stereotypical head-centred position with every echolocation call. Surprisingly, *P. discolor* can adjust the timing and the magnitude of these ear movements to the distance of the reflectors with millisecond precision. Our findings reveal echolocation-specific specialisations as well as general principles of scanning and stabilisation of a directional remote sense. The call-correlated movements of the facial structures may lead to a higher directionality of the echolocation system and may enable the bats to adjust their echo-acoustic gaze to dynamic environments.

KEY WORDS: Ear movements, Pinna movements, Noseleaf movements, Directionality, Scene analysis, Active sensing

INTRODUCTION

For orientation, animals face the challenge of exploring environments that are usually very complex. When an animal is navigating, movement additionally introduces dynamic changes in its environment. These, in turn, result in changes in the objects' relative position, are analysed by the animal's sensory system and thus produce sensory flow. Sensory flow is the change of sensory information elicited either by the movement of an object in a sensory scene or, more commonly, by motion of the observer. One feature that is common to many sensory systems is their directionality. Directionality allows the sensitivity for stimuli from a certain point in space to be enhanced while the sensitivity for stimuli originating from other locations is decreased. This, however, requires that larger volumes are investigated successively, i.e. that larger volumes are scanned.

In the human visual system, for example, spatial resolution is poor in the periphery, but high in the centre (Yarbus, 1967). If visual details are required, the fovea, the region on our retina that produces our sharp central vision, needs to be directed towards the target of interest. As a result, the exploration of new stimuli triggers saccadic eye movements (cf. for example Kandel et al., 2000; Yarbus, 1967). Saccades are fast, dart-like movements of the eyes that are employed for foveal scanning, i.e. to change the points of fixation (Lamansky, 1869; Müller, 1826). They occur upon the exploration of stationary scenes to obtain details for different points in space. In dynamic situations, when an observer moves relative to the environment, saccades arise as different structures are scanned successively (cf. Yarbus, 1967).

Scanning occurs not only in the visual system but also in the auditory system of animals with large, motile ears: in cats, an orienting response that involves movements of both the eyes and the motile ears is initiated, when the cats orient towards an auditory or visual stimulus (Populin and Yin, 1998). The allocentric orientation of the ears remains locked while the cats readjust their head position to face the direction of interest (Tollin et al., 2009).

Bats are acoustically guided animals that mostly rely on echolocation for navigation and orientation. They emit echolocation calls through the mouth or the nostrils and receive returning echoes via their outer ears. Both the emitters and the receivers feature a high directionality, they are motile, and they can be moved independently from one another (Aytekin et al., 2004; De Mey et al., 2008; Firzlaff and Schuller, 2003; Jakobsen et al., 2013; Obrist et al., 1993; Vanderelst et al., 2010).

This raises the question whether there exists an echo-acoustic counterpart to the scanning movements employed by the visual system for foveal scanning. As echolocation is an active sense, whose overall directionality is the product of the sender and receiver directionality, scanning could be achieved by both the emitting system and/or the receiving system.

Movements of the emitter are conceivable for bats emitting through the mouth (Kounitsky et al., 2015) or through a motile noseleaf. Indeed, there have been observations of noseleaf movements in rhinolophid bats. It was shown that both the lower and upper part of the noseleaf can move, accompanying call emission (Feng et al., 2012; He et al., 2015). However, there is to our knowledge only anecdotal evidence of noseleaf movements in one species of phyllostomid bats, *Macrophyllum macrophyllum* (Weinbeer and Kalko, 2007); an experimental investigation of noseleaf movements in phyllostomid bats is missing.

Scanning via the receivers is also apparent. Conspicuous ear movements in rhinolophid and hipposiderid bats were discovered many years ago (Möhres, 1953; Möhres and Kulzer, 1956; Schneider and Möhres, 1960). Both bat groups produce alternating ear movements when echolocating (Griffin et al., 1962;

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Möhres, 1953; Pye and Roberts, 1970). More recent studies report ear movements in echolocating fruit bats (Holland and Waters, 2005) and in verspertillionid bats (Wohlgemuth et al., 2016).

Here, we formally investigated how phyllostomid bats employ facial accessory structures of their echo-acoustic system for the echo-acoustic analysis of their environment. We hypothesised that phyllostomid bats move their facial features to adaptively modulate the directionality of signal emission and reception. If this hypothesis is true, we would expect to see changes in the shape of the noseleaf and position of the ears that coincide with the emission of echolocation calls. Consequently, we specifically addressed the question how bats of the species *Phyllostomus discolor* time the movements of their facial structures with respect to the timing of their sonar emissions and echo reception.

Our previous work (Kugler et al., 2016) has revealed significantly different flight manoeuvres of these bats, dependent on lateral structures: when bats flew between two lateral wall reflectors, their flight paths were aligned to the midline between the wall structures when the walls carried the same ridge orientation. When one wall carried vertical and the other horizontal ridges, bats consistently flew closer to the horizontal ridges that produce weaker echo-acoustic flow. We therefore hypothesised that the bats adjust movements of their facial features to the ridge orientation of lateral wall reflectors, e.g. by producing saccade-like ear movements serving to inspect a vertically ridged wall more closely. Consequently, in our first experiment, we tested whether our bats adjust movements of their facial features to the ridge orientation of laterally presented ridged walls.

It is well known that bats reduce both call level and duration when approaching a target (Aytekin et al., 2010; Griffin, 1958; Moss and Surlykke, 2010; Neuweiler, 1989; Schnitzler and Kalko, 2001). Here, we aimed to find range-dependent adjustments of the movements of facial features that accompany adjustments in echolocation behaviour. Therefore, in the second experiment, we placed large column reflectors at the end of the bats' track.

MATERIALS AND METHODS

To address these questions, we needed to monitor both a close-up of the facial structures, including the motile noseleaf and outer ears, and the sonar beam of the bat. Measuring facial movements in flying bats is very difficult because it requires high-resolution, high-speed stereo videos over a fairly long distance. To overcome these difficulties, bats were secured in a cart mounted with a high-speed camera and microphones at a constant distance from the bat's face. The cart could be moved along a track to stimulate the bat to echo-acoustically inspect its surroundings, even when not flying.

Experimental setup

The setup consisted of a cart (see Fig. 1A) that was driven along a 6 m long linear rail (ITEM Industrietechnik GmbH, Solingen, Germany). The cart was moved via a geared belt drive and a motor (DC Servomotor Serie 3268 BX4 AES, Dr Fritz Faulhaber GmbH & Co. KG, Schönaich, Germany) that was controlled by a computer. The position of the cart was recorded at a sampling rate of 10 Hz. The bat was positioned in a holder on the cart, mounted on a ramp (25 deg slope), which kept the animal's body in a steady position while its head and ears remained motile. The holder was stuffed with soft foam to avoid injury to the bats, covered with tissue adhesive tape for easy cleaning and lined with exchangeable cloth. It was composed of two half-shells that were sealed by hook-and-loop fasteners to prohibit the animal from escaping. On each side, a pole in the front part of the holder was placed between the head and the respective wing. Hence, the animal could not escape to the front.

A hemi-circular microphone array consisting of seven level-calibrated, ultrasonic microphones (custom built from SPU0410LR5H, Knowles Electronics, Itasca, IL, USA) with an angle of 30 deg to one another surrounded the head of the animal at a distance of 6 cm. Audio signals were preamplified (three microphones via Quadmic, RME Audio AG, Haimhausen, Germany; four microphones directly via the audio interface) and A/D converted with a sampling rate of 192 kHz by the audio interface (Traveler, MOTU, Cambridge, MA, USA).

Synchronised stereo videos were recorded under infrared illumination with a high-speed camera (Gazelle GZL-CL-22C5M-C, Point Grey Research Inc., Richmond, Canada) via two mirrors. The mirrors were installed at an angle of 130 deg to each other and inclined to the horizontal plane by 35 deg. The camera was deflected 10 deg from the horizontal plane. The images were mono- and stereo-calibrated with the Camera Calibration Toolbox for Matlab (Jean-Yves Bouguet, California Institute of Technology, Pasadena, CA, USA). For synchronisation of audio and video, the camera was triggered at a frame rate of 230 frames s^{-1} by the audio interface. The delay between video and audio was determined and taken into account for the analyses. Video data were transferred to the computer via a grabber (Xcelera-CL PX4 Full, Teledyne DALSA, Waterloo, Canada).

Two infrared light sources (custom built from Osram SFH4716S, Osram GmbH, Munich, Germany; with focusable lens system) flanking the camera on both sides were set to illuminate the bat's ears and noseleaf evenly.

Reflectors

We define reflectors as objects in the surroundings that the bat can detect using echolocation while in the apparatus. We used two types of reflectors: wall and column reflectors. The wall reflectors flanked the test section, constituting elongated structures which produced a relatively constant sensory flow along their entire length (see Fig. 1B). The column reflectors were placed at a fixed point in space, shortly before the end of the bats' track (see Fig. 1C). While the bat was moved towards the columns, we expect their reflections to decrease in delay and increase in amplitude and azimuth, in a manner typical for sensory flow.

Ridged wall reflectors

Reflectors for this experiment (see Fig. 1B) were the 3 m-long, structured side walls along the test zone. Each wall was planked vertically on one side and horizontally on the other with tongue-and-groove panelling, which created periodic ridges and grooves. The width of the ridges was 7.5 cm; the width of the grooves was 1.5 cm. By rotating the side walls, we could change the orientation of the ridges between horizontal and vertical for each side wall in the test zone. The walls flanked the path of the bat symmetrically and could be positioned at one of four possible lateral distances to the bat's trajectory (10, 15, 30 and 60 cm).

Column reflectors

Reflectors for this experiment (see Fig. 1C) were hard plastic pipes with a diameter of 16 cm and a height of 50 cm. The reflectors were placed 3 m from the starting point and approximately 2 m from the point where the cart reached a constant speed of 1.2 m s^{-1} (the speed at which it was driven towards the reflectors). The columns were presented either only on one side of the rail or symmetrically on both sides. Columns were presented at a lateral distance of either 8 or 22 cm.

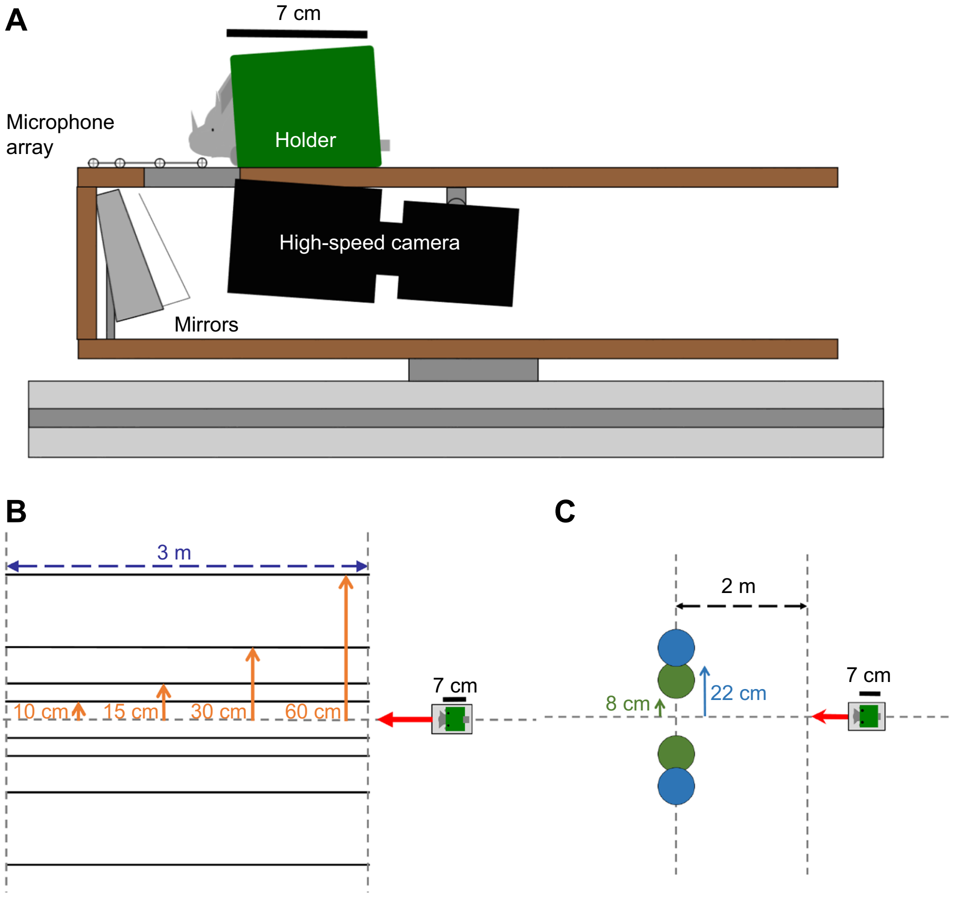

Fig. 1. Experimental setup. (A) Schematic diagram of the cart carrying the holder with the bat as well as the recording equipment. Underneath the bat, we installed the high-speed camera, which was directed onto the bat's face via two mirrors that were directed towards the animal at two different angles. This allowed us to record perfectly synchronised stereo videos of the bat's face. To both sides of the camera, an infrared light with focusable lenses was set to produce videos with even illumination (not shown). A hemicircular microphone array consisting of seven microphones, spaced by 30 deg, each at 6 cm from the animal, was used to record the animal's vocalisations. The cart was moved along a rail by a geared belt drive that was controlled via the computer. (B) Experimental setup with 3 m-long wall reflectors flanking the test section. These were positioned symmetrically around the bats at lateral distances of 10, 15, 30 or 60 cm. Wall reflectors carried ridges that were oriented vertically on one side and horizontally on the other, such that the orientation could be changed by rotating the reflector around its axis. (C) Experimental setup with column reflectors positioned 2 m after the point where the cart reached a constant speed. The column reflectors could be presented either only on one side or on both sides. The lateral distance of the column reflectors was either 8 or 22 cm.

Procedure and data acquisition

Each bat underwent a 2 week adaptation period in which it was familiarised with the handling procedure and the holder. Data acquisition took place a maximum of 5 experimental days later. In case this period did not suffice for data acquisition, a second data acquisition period followed after a minimum of 3 weeks.

For data acquisition, white markers (small blobs of Tipp-Ex ECOlutions Aqua, Clichy Cedex, France) were placed at four specific positions on the experimental animal's face: at the base and at half-height of the noseleaf and at half-height of the frontal rim of each ear (see Figs 4A and 5A; Movie 1). Then, the bat was placed in the holder. The stereotyped movement of the cart was initiated by the experimenter: the cart accelerated to a maximum speed of 1.2 m s^{-1}, was driven along the test zone and decelerated. The movement was controlled via the computer. A 4.5 s audio and cart movement ringbuffer was saved with Matlab (MathWorks, Natick, MA, USA) and the soundmexpro (HörTech, Oldenburg, Germany) audio toolbox. Video data from the same period were recorded with StreamPix 5 (NorPix, Inc., Montreal, QC, Canada) into a video ringbuffer. During the adaptation period as well as data acquisition, the animal spent a maximum of 5 min per day in the holder.

Analysis

Trials were excluded from the analysis when the animal displayed evasive behaviour or did not produce echolocation calls. For each trial, only frames where the acceleration of the cart was less than 0.5 m s^{-2} and the driving speed exceeded 0.8 m s^{-1} were analysed.

Video analysis

Video analysis was carried out in multiple steps. First, the stereo images were split into two image stacks. Each of these was preprocessed with ImageJ (National Institutes of Health, Bethesda, MD, USA) to extract the 2D coordinates of the markers from each image, as follows: background subtraction using a sliding paraboloid algorithm was applied; then, a threshold was determined to transform the greyscale image to a binary image that ideally contained only the markers; finally, noise was removed using the ImageJ function 'noise despeckle'.

The subsequent steps were all performed using custom-written Matlab programs: the markers were tracked over time in a semi-automated manner. A 3D reconstruction based on epipolar geometry was performed for each marker, using the stereo_triangulation function from the Camera Calibration Toolbox.

To determine whether the timing of noseleaf movements was correlated with echolocation calls, we calculated averages of the noseleaf movements as follows. First, the distance between the markers at the base and at half-height of the noseleaf was calculated and interpolated at the audio sampling rate. The values in the window −80 ms to 100 ms re. call emission were cut out for each call that was not preceded by another call for at least 70 ms. For each trial, an average over all these movements was calculated. For the bending noseleaf movements, the time when the noseleaf distance reached a minimum 10 ms before call emission or later was extracted from the average to determine when the noseleaf was maximally bent.

For the call-correlated ear movements, the distance between the markers at half-height of the frontal rim of the ear was calculated and interpolated at the audio sampling rate. The values in the window −60 ms to 90 ms re. call emission were cut out for each call that was not preceded by another call for at least 70 ms. For further analyses, the time point when the ears were maximally raised, i.e. when the distance between the two ears started to increase again in the window −35 ms to 30 ms re. call emission, was determined for each single call. For every trial, an average over all movements, for which all coordinates in the analysis window could be determined, was calculated.

Pearson correlations between the reflector distance and the time when the ears were maximally raised were calculated. For the data with the ridged wall reflectors, we calculated correlation coefficients and *P*-values between the lateral distance of the reflectors and the time when the ears were maximally raised. For the data acquired with the column reflectors, we calculated for each call the direct distance between the animal and the reflectors, separately for every animal and reflector arrangement. Correlation coefficients and *P*-values were determined between the direct distance between the animal and the reflectors and the time when the ears were maximally raised. For these calculations, we chose for each dataset the experimental condition that we estimated would produce the highest alertness in our bats: with wall reflectors, we chose trials acquired with vertical ridge orientation on both sides, as this constitutes reflectors with the highest target strength; for column reflectors, we chose the experimental condition with symmetrically arranged columns at 8 cm lateral distance, as this constitutes a narrow passage, narrower than the animal's wingspan, and thus would require the animal to retract its wings during flight.

For the same reasons, we used these same data subsets of trials for the next analysis: Pearson correlations between the minimal ear–ear distance and the distance to the reflectors were computed for each animal. For the data acquired with wall reflectors, we calculated correlation coefficients and *P*-values between the lateral distance of the reflectors and minimal ear–ear distance. For the data acquired with column reflectors, we calculated correlation coefficients and *P*-values between the direct distance between the bat and the column reflectors at the time of call emission and the distance between the markers on the ears at the time when the ears were maximally raised. Call-correlated noseleaf and ear movements were observed in all individuals from which data were obtained.

Audio analysis

All audio analyses were done in Matlab with custom-written programs. Amplitude-based call detection was carried out on recordings that had been high-pass filtered at 35 kHz. The microphone on which the call was recorded with the highest amplitude was determined and the following analysis steps were carried out using the respective recording. The onset and offset of calls were determined as the time points when the envelope of the rectified recording exceeded and fell below an amplitude threshold, respectively. The duration was calculated as the time between onset and offset of the call. We measured the latency of facial movements relative to the time point of the maximal call amplitude. Call level was calculated in decibels within a fixed 4 ms time window centred on the maximal call amplitude. The descriptive statistics for all analysed parameters are given as medians (with first and third quartiles).

Animals

The experimental animals were 6 adult specimens of the lesser spear-nosed bat, *Phyllostomus discolor* Wagner 1843. Data were recorded for 3 individuals (2 males, 1 female) with the ridged wall reflectors and for 5 individuals (3 males, 2 females) with the column reflectors. Two of the bats (1 male, 1 female) participated in both experiments. *Phyllostomus discolor* is a neotropical bat species that feeds on fruit, nectar, pollen and insects in a forest habitat (Kwiecinski, 2006). Hence, this species has to navigate through highly structured surroundings. *Phyllostomus discolor* emits brief (<3 ms) broadband multi-harmonic echolocation calls covering the frequency range between 45 and 100 kHz (Rother and Schmidt, 1982). All experiments were conducted under the principles of laboratory animal care and the regulations of the current version of the German Law on Animal Protection. Experiments were approved by the Regierung von Oberbayern (55.2-1-54-2532-38-2014). Approval to keep and breed the bats was issued by the Munich district veterinary office.

RESULTS

Here, we will first present evidence that our bats used echolocation quite naturally despite being restrained in the moving cart. Second, we will describe the facial movements of both the noseleaf and the ears in detail, and finally, we will investigate how these movements depend on the reflectors presented.

Our bats adjusted call parameters to their surroundings, similar to bats navigating in the wild (Aytekin et al., 2010; Griffin, 1958; Moss and Surlykke, 2010; Neuweiler, 1989; Schnitzler and Kalko, 2001). Specifically, our bats adjusted call duration to the lateral distance to the flanking walls to avoid overlap between returning echoes: call duration typically decreased with decreasing distance of the lateral walls, except for bat 3, which did not consistently shorten calls for closer walls (see Fig. 2A) and also did not adjust call level (data not shown). Likewise, 4 out of 5 bats (except bat 1) shortened call duration while approaching the column reflectors (see Fig. 2B). We checked whether bat 1 adjusted other call parameters and found that this bat significantly reduced the call level over the last metre before passing the column reflectors (data not shown). Overall, these basic audio data confirm that the bats displayed a quite natural echolocation behaviour although they were fixed in a moving holder and not in free flight.

Despite the simple linear movement of the bats along the reflectors, sonar beam reconstructions from the microphone-array recordings show that the bats point their beam quite variably in azimuth: beam pointing is illustrated in Fig. 3. The data show that while the beam is on average pointed well to the front, standard deviations are quite large (of the order of ±20 deg), indicating that the bats point their beams quite variably left and right. Inspection of the corresponding video footage confirmed that this was due to pronounced lateral (yaw-type) scanning head movements.

Sonar emission behaviour of the bats was always accompanied by conspicuous movements of the bats' facial structures. Measurements of the distance between the markers (see Figs 4A and 5A) as a function of time (see Figs 4D and 5C) revealed call-correlated movements of both the noseleaf and the ears. An example stereo movie with accompanying sound recording and 3D reconstruction is provided in Movie 1. The call-correlated movements of the noseleaf usually showed a stereotyped pattern: the noseleaf tensed before a call and relaxed thereafter. Tensing of the noseleaf could lead to the noseleaf either bending or stretching in shape. To quantify these impressions from the video footage, we measured the distance between the two markers on the noseleaf (see Fig. 4A): an example trace of noseleaf movements is shown in Fig. 4D; the distance between the markers as function of time to call emission is shown in Fig. 4B. When the noseleaf bends, the distance between the markers decreases; when the noseleaf stretches, the

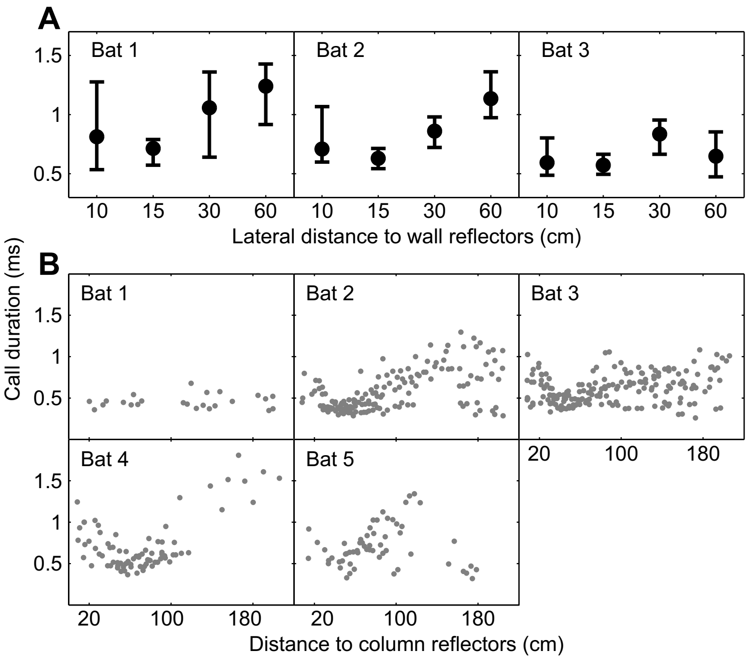

Fig. 2. Analysis of the duration of echolocation calls dependent on reflector distance. (A) For the experiment with wall reflectors, call durations for all calls an animal emitted within vertically orientated walls were pooled for each lateral reflector distance (10, 15, 30 or 60 cm). The graphs show the median with the first and the third quartile as error bars. Overall, call duration decreased with decreasing distance to the lateral walls. (B) For the experiment with column reflectors, the distance between the bats and the column reflectors decreases continually as the bat is driven along the rail. Call duration is plotted against the direct distance between the bat and the column reflectors, determined for each call. Again, data show that overall call duration decreased with decreasing distance of the bats to the reflectors. Bat 1 did not adjust call duration, but clearly reduced call level in the last metre before the column reflector (data not shown).

distance increases. The data show that the movement starts about 35 ms before call emission and lasts until about 70 ms after emission. This time course is similar for the dominant bending movement and the less frequent stretching movement. The percentage of these different noseleaf movements is illustrated in Fig. 4C. Fig. 4E shows that bending of the noseleaf is maximal about 5–10 ms after call emission.

Call-correlated movements of the bats' ears likewise conformed to a stereotyped pattern: for each call, both ears were raised and set in an upright position pointing both ears towards the front, before the tension was released again. Raising of the ears led to a decrease in the distance between the ear markers (see Fig. 5A). The release of the tension caused a downward movement of the ears to a more lateral and suspended position. This downward movement was often omitted when the call was the first in a group, i.e. when the following call occurred within the next 35 ms. Again, these movements were quantified as distance measures between the two markers on the bats' ears. An example trace of the distance is shown in Fig. 5C. Fig. 5B shows how the distance between the ears changes as function of time to call emission. The data show that the ears start to rise about 35 ms before call emission and that this ends about 45 ms after emission. Ears are maximally raised about 2–8 ms after call emission (Fig. 5D).

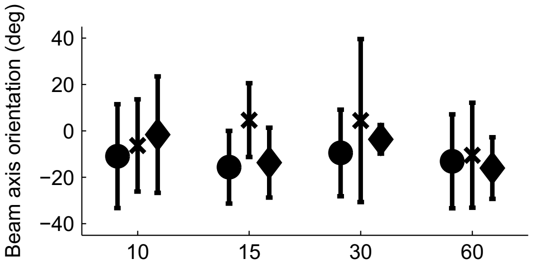

Fig. 3. Beam axis orientation for different lateral reflector distances while bats are moved between the wall reflectors. The beam axis orientation is defined as the direction in which the bats point their sonar beam. Negative values indicate beam orientations towards the left, positive values indicate those to the right. Different symbols represent mean beam orientations of individual bats for different reflector distances; error bars represent standard deviations. Data show that while bats on average pointed their beams to the front, beam pointing was quite variable but did not depend on the distance of the lateral reflectors.

Next, we checked whether call-correlated ear movements depended on the reflectors and the bat's position towards them. Our previous work (Kugler et al., 2016) has revealed significantly different flight manoeuvres of these bats, dependent on lateral structures. However, we found only two instances where the animals' adjusted movements of their facial features depended on the spatial arrangement of the lateral reflectors. These two instances are reported below.

We investigated whether the time when the ears were maximally raised correlated with the distance to the reflectors. For each dataset, we chose the experimental condition that we assumed to cause the bat to be most alert. With our wall reflectors, we analysed trials acquired with vertically ridged reflectors, as they produce the loudest echoes, which vary with the relative position of the animal to the ridges. When the bats were moved between the vertically ridged walls, we found that the latency of raising the ears after call emission was shorter when the walls were closer (see Fig. 6A). This was significant in two out of three bats. For data acquired with column reflectors, we chose the symmetrical arrangement of reflectors at the closer lateral distance of 8 cm as this is a passage that is narrower than the bats' wingspan. The results showed the same qualitative trend as with the wall reflectors: in three out of five bats, the latency of raising the ears was significantly longer when the bats were further away from the reflectors and shorter when the distance to the reflectors was shorter. Correlation coefficients and *P*-values for correlations between the distance to the column reflectors and the time when the ears are maximally raised are shown in Fig. 6B. We then checked whether the magnitude of the ear movements changed with distance between the bat and the reflectors. Therefore, we analysed the distance between the ears at different reflector distances (see Fig. 7). Most bats appeared to raise

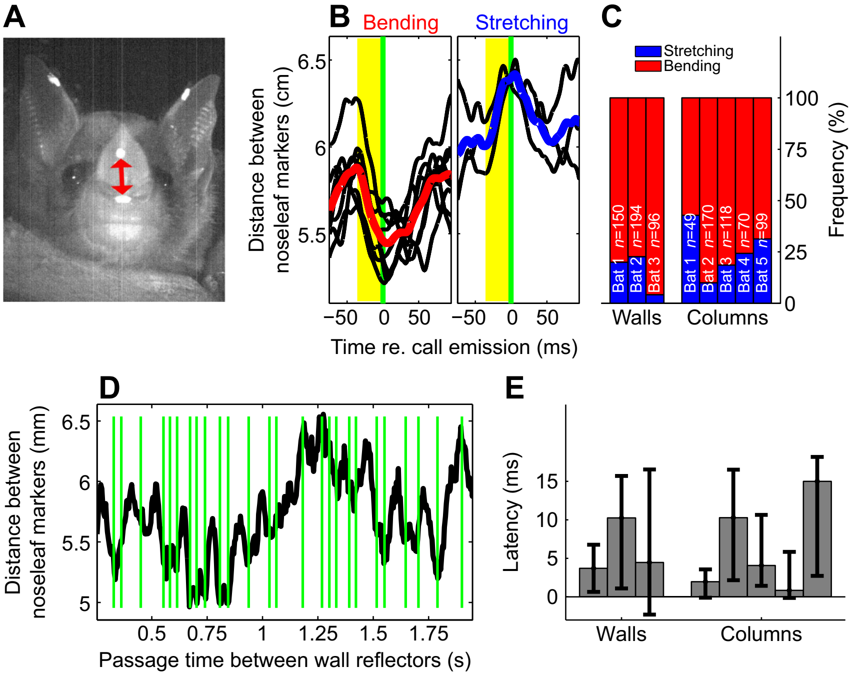

Fig. 4. Call-correlated movements of the noseleaf. (A) A bat performing the experiment. Markers were placed at the base and at half-height of the noseleaf and at half-height of the frontal rim of each ear. A movement of the noseleaf is quantified as a change in distance between the two markers (red arrow) on the noseleaf that either move towards or away from each other when the bat bends or stretches its noseleaf, respectively. (B) The distance between the two noseleaf markers in a fixed time window around call emission. The green line indicates the time of call emission. The yellow bar highlights the time window −35 to 0 ms re. call emission. The slope of the movement trace in this temporal window was used to determine whether a movement was a bending or a stretching movement (if values decrease, it is a bending movement; if they increase, it is a stretching movement). Averages are depicted as the bold red and blue line for bending and stretching movements, respectively. Most call-correlated noseleaf movements were accompanied by a bending of the noseleaf (left). (C) Frequency of noseleaf bending/stretching for all calls analysed for each of our bats. (D) Time course of the distance between the noseleaf markers for the respective trial. Green lines indicate the emission of echolocation calls. (E) The latency of the time to maximum bending of the noseleaf relative to the time of maximal call amplitude. Bars represent medians of individual bats; error bars show interquartiles.

their ears more when the reflectors were closer. This effect was significant for bat 2 with the wall reflectors and for bats 2, 3 and 5 with the column reflectors. Overall, these data indicate that the call-correlated ear movements of the bats are not stereotyped motor programmes but depend on the echo-acoustic layout of the environment.

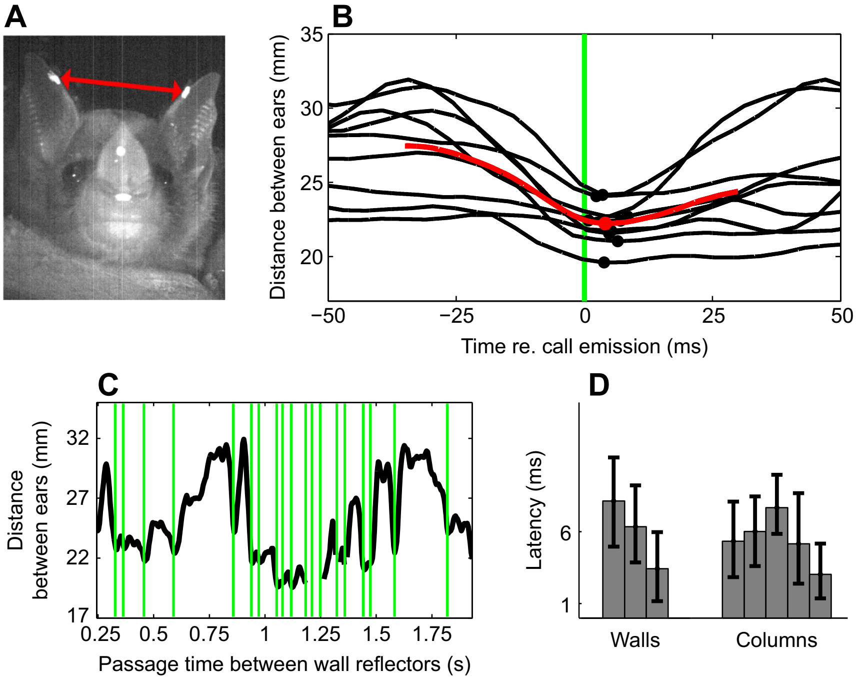

Fig. 5. Call-correlated ear movements. Same format as in Fig. 4. (A) The distance measure taken to quantify ear movements. The distance decreased when the bats raised their ears and increased when the ears moved down and sideways. (B) The distance between the two ear markers in a fixed time window around call emission. The average is depicted as the bold red line. Bats always raised their ears for call emission and let them fall down and sideways thereafter, unless further calls followed closely after. (C) Time course of the distance between the ear markers. Green lines indicate the emission of echolocation calls. (D) The latency of the time to maximum raising of the ears relative to call emission. Bars represent medians of individual bats; error bars show interquartiles.

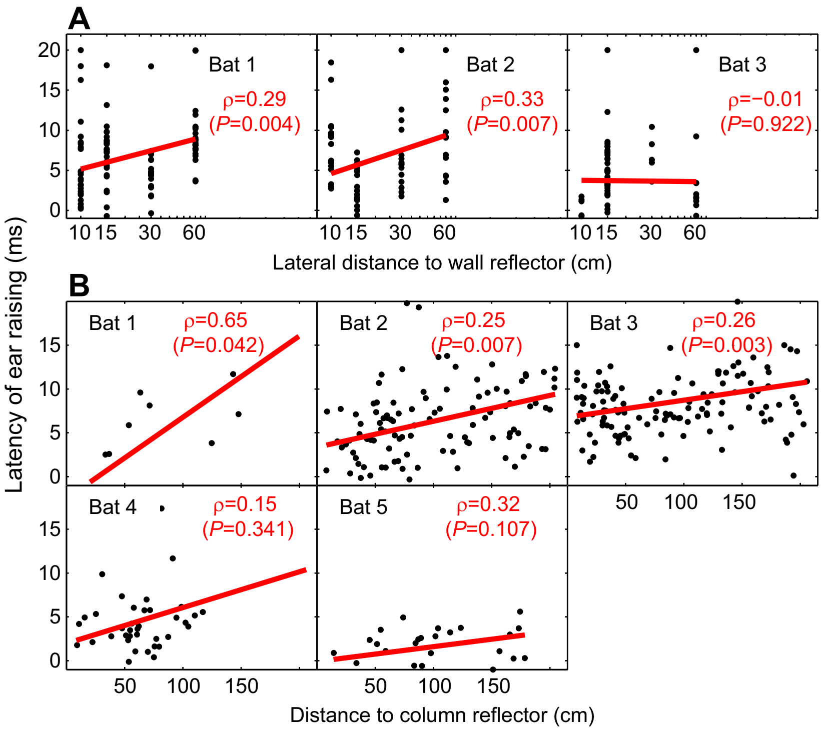

Fig. 6. Correlation between the latency of ear raising and distance to reflectors. (A) Lateral distance to the wall reflectors and the latency of ear raising. Bats 1 and 2 both raised their ears significantly earlier when the walls were closer. (B) Direct distance to the column reflectors and latency of ear raising for each call. All bats tended to raise their ears earlier when they were closer to the column reflectors. The effect was significant for bats 1–3. Correlation coefficients for a Pearson correlation (ρ) and *P*-values are provided in red for each plot.

DISCUSSION

The novel bat cart setup allowed for very accurate 3D reconstruction of facial structures on the move: we were able to monitor noseleaf and ear movements as well as echolocation behaviour while moving the bats along different lateral reflectors. In our experiments, most bats adjusted their echolocation behaviour according to their distance to the reflectors by reducing call duration when closer reflectors caused the risk of echo overlap. This is in line with previous observations (Aytekin et al., 2010; Griffin, 1958; Moss and Surlykke, 2010; Neuweiler, 1989; Schnitzler and Kalko, 2001). Our results also reveal that the characteristic noseleaf and ear movements that had been reported anecdotally in previous publications are precisely timed with call emission, even though a previous paper claimed otherwise for phyllostomid bats (Pye and Roberts, 1970). However, there remains plasticity in this behaviour, as the bats are able to adjust

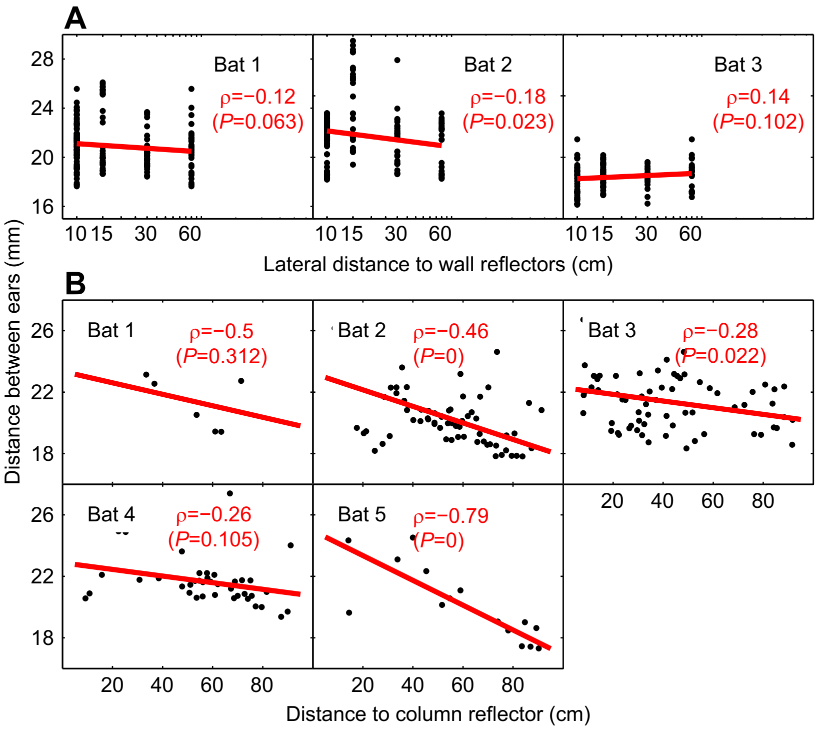

Fig. 7. Correlation between magnitude of ear movement and distance to reflectors. The magnitude of ear movement was expressed as the distance between the ears. (A) Lateral distance of the wall reflectors and the minimal distance that was detected for the respective ear-raising movement. Bats 1 and 2 both raised their ears more when the walls were closer, but this was significant only for bat 2. (B) Direct distance between the bat and the column reflector, determined for each call. All bats raised their ears less when they were closer to the column reflectors. This effect was significant for bats 2, 3 and 5. Correlation coefficients for a Pearson correlation (ρ) and *P*-values are provided in red for each plot.

both the timing and the magnitude of the ear movements according to their distance to the reflectors.

The observed noseleaf movements manifest mainly as a movement of the upper part of the noseleaf, the lancet, which resulted in most cases in a bending of the noseleaf. Movements of different parts of the noseleaf have previously been described in rhinolophid and hipposiderid bats. The lancet movements, in particular, have been predicted to considerably alter the call emission patterns, for the most part in elevation (Gupta et al., 2015; He et al., 2015). Some studies have addressed the question whether a movement of the noseleaf would also alter the emission pattern in phyllostomid bats (Hartley and Suthers, 1987; Vanderelst et al., 2010). These studies postulate that the directionality of the call emission pattern along the horizontal plane is mostly determined by the spacing between the nostrils as it arises as a result of interference of the emission from the two nostrils, i.e. two single sound sources. The authors (Hartley and Suthers, 1987) predict that a displacement of the lancet would cause changes in the emission pattern mainly in elevation. In a computational approach, Vanderelst et al. (2010) confirm these findings, but show that removal of the lancet would only negligibly affect the combined directionality of the emitting and the receiving system. However, their simulation reveals that bending the noseleaf forward by as little as 10 deg significantly lowers the sonar beam. Hence, there is reason to believe that the noseleaf movements we observed in *P. discolor* serve to steer the echolocation beam in elevation. Unfortunately, the current horizontal microphone array did not allow us to quantify sonar-beam pointing in elevation.

Our bats produced call-correlated ear movements. When a bat emitted an echolocation call, both ears were raised to an upright, frontal orientation. After a call or call group, the ears lowered to a more lateral and suspended orientation. Ear movements have so far been reported in a number of bat species, including rhinolophid and hipposiderid bats as well as vespertillionid and phyllostomid bats (Griffin et al., 1962; Holland and Waters, 2005; Möhres, 1953; Möhres and Kulzer, 1956; Pye and Roberts, 1970; Schneider and Möhres, 1960; Weinbeer and Kalko, 2007; Wohlgemuth et al., 2016). Depending on the way a bat species echolocates, ear movements can have very different perceptual effects. In terms of echolocation call design, bats can be divided into two main groups: CF bats produce signals with constant frequency tones that are usually rather long (several tens of milliseconds); FM bats, in contrast, produce short (<20 ms), frequency-modulated broadband calls.

Most studies reporting echolocation-related ear movements in bats were carried out with CF bats, e.g. rhinolophid and hipposiderid bats (Gao et al., 2011; Griffin et al., 1962; Mogdans et al., 1988; Möhres, 1953; Pye and Roberts, 1970; Schneider and Möhres, 1960). The ear movements of rhinolophids and hipposiderids conform to the same pattern: the bats move one ear forward, straighten and rotate it such that its opening faces forward; simultaneously, the other ear is pulled backward into a more relaxed posture that causes it to turn towards the side. This process is alternated between the ears. The ear movements occur on a similar time scale to the echolocation calls and are roughly, but not perfectly, synchronous to the echolocation calls (the alternation rate is about half the call rate; Gao et al., 2011; Griffin et al., 1962; Pye and Roberts, 1970). It has been shown that these ear movements play a major role in echo-acoustic target localisation in rhinolophid bats: immobilisation of the outer ears caused a decrease of localisation performance, especially in elevation (Gorlinsky and Konstantinov, 1978; Mogdans et al., 1988; Schneider and Möhres, 1960).

The way an incoming sound is changed by the ears depends on both sound frequency and the angle of incidence. These dependencies can be used for localisation in elevation. CF bats work with a single dominant frequency; thus, they cannot evaluate changes as a function of frequency. This limitation is overcome by the animals making strong, alternating ear movements, analysing both monaural and binaural amplitude changes induced by the movements, and deducing target elevation from these dynamic monaural and binaural changes (Vanderelst et al., 2015; Walker et al., 1998). FM bats, in contrast, emit a broad range of frequencies almost simultaneously. Like other mammals trying to localise a sound source in elevation, they can analyse changes in the echo as a function of frequency and deduce target elevation without the need to monitor changes as a function of time during ear movements (Carlile and King, 1994; Middlebrooks and Green, 1991; Roffler and Butler, 1968; Tollin and Yin, 2003). We argue that this is the reason why our bats can afford to orient their ears quite stereotypically to the front while echolocating. Nevertheless, it is likely that FM bats will also profit from head and ear movements in a vertical localisation task (Chiu and Moss, 2007; Hoffmann et al., 2015; Lawrence and Simmons, 1982), as is true for other mammals.

The question arises why we observe ear movements in our FM bats, if these are not even required for localisation because the bats could simply hold their ears still in a default position. A possible explanation can be found in a study by Holland and Waters (2005) that reports ear movements in flying foxes (*Rousettus aegyptiacus*) which echolocate using tongue clicks. These animals move both ears forward as they produce their short echolocation signals. Subsequently, both ears are moved backwards again. This pattern of ear movements synchronised with echolocation is similar to what we describe here in *P. discolor*. Holland and Waters (2005) hypothesise that by altering ear posture from a more insensitive (facing back) to the most sensitive (facing forward) posture, the animals can alter the sensitivity to returning echoes. This is conceivable as simulations have shown that in horseshoe bats the sensitivity to incoming sounds is more focused to a frontal region when the ears are in an upright position, facing forward, whereas sensitivity is less directional and broader when the ears are bent (Gao et al., 2011). Holland and Waters (2005) suggest that bats could prevent forward masking by smart timing of maximal sensitivity: by reducing the sensitivity of the receiving system until echoes from objects of interest return, early returning (clutter) echoes could be attenuated while the bats could still call at high intensities. Echoes from objects of interest could be optimally amplified as they would impinge on the ear when it is most sensitive. In our experiment, bats did raise their ears earlier with closer reflectors, supporting this hypothesis. Another advantage of preserving ear motility is that this allows orientation of the main axis of the ear in the direction in which a target is located. In our experiments with the column reflectors, bats raised their ears less when the angle to the columns was larger, thus orienting the main axis of the ear more laterally and broadening the sensitivity of the receiving system, which also allows for better perception of echoes from peripheral objects (Gao et al., 2011). Finally, ear motility could aid in passive sound localisation.

Between echolocation calls, our bats moved their ears consistently, often in an alternating manner, i.e. one ear moved to the front while the other ear moved to the side and vice versa. This behaviour is consistent with a passive-acoustic scanning, i.e. probing the environment for external sound sources. Given that our bats performed a quite stereotypical raising of the ears for each echolocation call or call group, we hypothesise that ear motility is preserved in *P. discolor* bats to allow switching between echo-acoustic and passive-acoustic scanning of the environment.

Based on our observations and previously established models on the effect of noseleaf and ear movements, we suggest that these ear and noseleaf movements interact in our bats, but have different functions. We suggest that *P. discolor* employs ear movements to adjust the sensitivity of the receiving system dynamically according to the needs imposed by the auditory scene. Noseleaf movements are more likely to aid in adjusting the sonar beam by redirecting sound to different elevations to the front of the bat and thus to scanning different regions in space (Reijniers et al., 2010).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: K.K., L.W.; Methodology: K.K., L.W.; Software: K.K., L.W.; Validation: L.W.; Formal analysis: K.K., L.W.; Investigation: K.K., L.W.; Resources: L.W.; Writing - original draft: K.K., L.W.; Writing - review & editing: K.K., L.W.; Visualization: K.K., L.W.; Supervision: L.W.; Project administration: L.W.; Funding acquisition: K.K., L.W.

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Supplementary information

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Highlighted Article: Echolocating bats move their noseleaf (for sound emission) and their ears for fast and directed echo-acoustic exploration of their surroundings.

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4 General discussion

The present thesis addresses the adaptive behaviour of echolocating bats in the context of echo-acoustic flow. To this end, two independent studies were conducted with the lesser spear-nosed bat *Phyllostomus discolor*. In an initial set of experiments, it was investigated if and how echo-acoustic flow is used by the bats as a cue for flight guidance when negotiating narrow passages. As bats use echolocation for navigation, spatial information about the environment is not directly available. As explained in section 1.3.1, in the visual system spatial information is projected onto the sensory epithelium, but in the auditory system spatial information about the environment needs to be computed. During flight, bats move quickly and hence need to be able to update information about the spatial arrangement of the environment fast enough to avoid obstacles and to coordinate manoeuvers for prey interception. So far, our understanding how bats cope with the challenge of quickly moving through complex environments is rather vague. Our study has revealed new insights into flight control in behaving bats by identifying echo-acoustic flow as one cue bats use for this task. A second set of experiments aimed to investigate how phyllostomid bats employ facial features of their echolocation system for the analysis of echo-acoustic flow. The information sensed by an animal crucially depends on the orientation and focus of the sensors during the acquisition of sensory information. In bats, the coordination of the emitting and the receiving echolocation system determines the overall orientation and focus of the echolocation system. In our second study, we monitored movements of the noseleaf and the pinnae as well as the general echolocation behaviour in bats that were exposed to echo-acoustic flow to tackle the question of how these bats scan space during movement.

4.1 Sensory flow as a cue used for the control of animal flight

In the flight experiments, the bats flew from one end of a flight tunnel with a width of 1.2 m to the other. While doing so, they were exposed to echo-acoustic flow in a 3 m long test zone. The echo-acoustic flow field in the test zone was altered in between trials. The structures that created the echo-acoustic flow were ridged walls with horizontal (weak echo-acoustic flow) and vertical (strong echo-acoustic flow) ridge orientation. The combined ridge orientation across the two side walls determines the strength of the overall echo-acoustic flow field: echo-acoustic flow is weakest within horizontally oriented ridges, intermediate when only one wall carries horizontal and the other vertical ridges, and highest when both side walls carry vertical ridges. In the first part of the experiment, the ridge spacing was larger than in the second part of the experiment. Consequently, the strength of the echo-acoustic flow created by the vertical ridges was higher in the second experiment.

Indeed, the bats showed flow field specific adaptations in their flight paths and in their echolocation behaviour. These behavioural adaptations will both alter the perceived echo-acoustic flow. The bats navigated away from a lateral surface that produces strong echo-acoustic flow. When doing so, the animal reduces the rate of change, and thus the flow strength from the respective side, in several parameters. The angular velocity of passing objects on the corresponding side wall as well as the changes in the distance to these objects are reduced. Hence, echo-acoustic flow on the corresponding side is weakened. The same effect would occur upon a reduction in flight speed. Flow field dependent reduction in flight speed was indeed observed in visually guided flyers (Bhagavatula et al., 2011; Dyhr and Higgins, 2010; Frye and Dickinson, 2007; Srinivasan et al., 1996). For example, budgerigars flew with mean speeds of ca. 4 and 6 ms^{-1} in strong and weak optic flow, respectively (Bhagavatula et al., 2011). However, in our experiments the bats did not significantly adjust flight speed to the strength of echo-acoustic flow. Instead, the animals flew at a rather stable flight speed of approximately 4 ms^{-1}. This means that visually and acoustically guided animals seem to behave differently with respect to flight speed adjustment to sensory flow. A reason for this might be ascribed to the very different sensing ranges of the visual and the echolocation system. The prey detection range for bats was estimated to lie between 2 and 10 m (Madsen and Surlykke, 2013). Larger objects produce stronger echoes and can be detected over larger distances. Stilz and Schnitzler (2012) calculated detection ranges for extended structures (like forest edges) for different bat species and different air conditions. Estimates of detection ranges for such large structures lie between 2.4 m (for phyllostomid bats at 100 % air humidity) and 47.7 m (for *Nyctalus lasiopterus* at 18 % air humidity). Under favourable conditions the range of the visual system can extend over several hundreds of meters for the same structures. When sensing range is large with respect to propagation speed, as usually applicable for vision, obstacles will be detected early enough to execute a successful evasive manoeuver. For echolocating bats, sensing range is potentially much shorter. Therefore, flying at high speed might present a serious danger for echolocating bats. Echo-acoustic sensing range could hence be a limiting factor for flight speed, especially when sensing ranges are as short as under tropical conditions. However, under our laboratory conditions, this was most likely not the case. Temperature and air humidity were not controlled for or set to a specific value in our experimental room, which was ventilated with ambient air. Therefore, temperature and air humidity were subject to fluctuations. Nevertheless, values surely did not reach tropical conditions, and most likely echo-acoustic sensing range exceeded the length of the flight tunnel (5 m in total).

In general, flight speed in bats can be quite variable and highly depends on the habitat and the feeding ecology of the bat species. Extreme flight speeds range from a standstill in hovering, nectar-feeding bats to the current record of 44.5 ms^{-1} in aerial hawking Mexican free-tailed bats (McCracken et al., 2016; Neuweiler, 2000). *Phyllostomus discolor* is an omnivorous, phyllostomid bat that forages in highly cluttered environments (Kwiecinski, 2006). Such bats usually fly rather slowly. Even though *Phyllostomus discolor* does sometimes feed on nectar, this species was predicted to fly relatively fast and be a poor hoverer due to its weight and wing morphology (Norberg and Rayner, 1987). More recent observations acknowledge *Phyllostomus discolor* to show intermediate levels of manoeuvrability and agility, but no ablility to hover (Giannini and Brenes, 2001). The authors observed that the bats land on a flower for feeding. For a smaller phyllostomid bat species, *Glossophaga soricina*, flight speed was found to depend highly on the length and the cross-sectional area of the flight corridor: measurements revealed a mean flight speed of 4.6 ms^{-1} in a 7 m long flight tunnel and 7.3 ms^{-1} for a 50 m long flight path (Winter, 1999). In general, flight speed is faster in larger than in smaller species (Norberg, 1995). Considering these facts, our recorded flight speed of approximately 4 ms^{-1} seems quite slow. Possibly this flight speed is so slow, that it would allow the bat breaking or manoeuvring early enough to avoid collisions. It is therefore conceivable that our bats would adjust flight speed, when flying at faster speeds that can only be reached in longer flight tunnels – and/or maybe only by faster flying bat species. A study by Warnecke et al. (2016) investigated navigation in big brown bats (*Eptesicus fuscus*) that were being exposed to echo-acoustic flow. The findings of this study are congruent with our findings: while bats shifted flight paths towards the side producing less echo-acoustic flow, the animals did not significantly adjust flight speed to echo-acoustic flow. Flight speed in big brown bats usually ranges between 2 and 6 ms^{-1} (Falk et al., 2014). In their 6.2 m long flight corridor Warnecke et al. (2016) recorded a relatively constant mean flight speed of ca. 3.8 ms^{-1} in all experimental conditions, a value that is comparable to the flight speed we recorded for *Phyllostomus discolor*, and likewise rather slow. Maybe both our study in *Phyllostomus discolor* and the study in *Eptesicus fuscus* did not allow the bats to reach flights speeds that are fast enough to be reduced under strong echo-acoustic flow. A previous experiment on flight and echolocation behaviour in environments with different levels of clutter (Falk et al., 2014) does hint towards bats adjusting flight speed to echo-acoustic flow: bats navigating through open space (producing weak echo-acoustic flow) fly faster than bats navigating through cluttered space (producing stronger echo-acoustic flow). However, this could simply represent a measure of precaution to an elevated collision risk in cluttered environments and not a reaction to echo-acoustic flow *per se*. As bats have direct access to object distance (via echo delay), an alternative strategy for flight guidance could be to orient along linear structures, like e.g. hedges, and keeping a stable lateral distance. Such behaviour has likewise been observed in a couple of bat species (Holderied et al., 2006; Limpens and Kapteyn, 1991), even though the disposition to cross open spaces instead of orienting along linear structures seems to vary among species (Limpens and Kapteyn, 1991). It remains an open question if bats adjust flight speed to echo-acoustic flow when conditions allow them to fly faster. Flight experiments analogue to ours (Kugler et al., 2016) and Warnecke et al. (2016) should be carried out in longer (and potentially broader) corridors, and with faster flying bat species to check if bats adjust flight speed under these conditions or if they rather orient along lateral structures.

4.2 Other cues likely to influence bat flight

Another surprising finding from the flight experiments was that our bats adjusted their flightpaths between the horizontally and the vertically ridged side wall to a similar amount with stimuli carrying the fine and the broad ridge spacing. This is striking, because vertical ridges create stronger echo-acoustic flow when carrying finely spaced ridges. Accordingly, one would expect a more pronounced flight path adjustment with stimuli carrying fine ridge spacing, if the bats were to guide their flight based solely on echo-acoustic flow. The fact that we did not observe this might indicate that echo-acoustic flow is not the only cue used by bats for guiding flights through narrow passages. As discussed in Kugler et al. (2016) bats have explicit information on their distance to nearby objects via echo delay. In theory, the animals could perfectly navigate in narrow spaces using echo-delay combined with the remaining information about the three-dimensional spatial layout of the surroundings. This would be sufficient to avoid colliding with objects. However, we could clearly show that echo-acoustic flow modulates flight guidance. The fact that echo-acoustic flow affects flight guidance is supported by neurophysiological data. Echo delay is encoded at different stages in the auditory pathway from the midbrain up to the auditory cortex (Mittmann and Wenstrup, 1995; O'Neill and Suga, 1979; Wenstrup and Portfors, 2011). Whereas neurons in the midbrain are arranged tonotopically, i.e. according to their frequency sensitivity, neurons in the posterior dorsal field of the auditory cortex are arranged according to target range, i.e. according to the call-echo delay they are most responsive to (the best delay). Recently, Bartenstein et al. (2014) discovered, that this cortical map can be dynamically modified. When they presented ecologically meaningful call-echo sequences simulating the bat approaching and passing a lateral object, response properties in the target range map changed: the area dedicated to represent close-range targets increased with decreasing lateral passing distance of the object. The authors conclude that "neurons in the auditory cortex [...] encode echo-acoustic flow information on the geometric relation between targets and the bat's flight trajectory, rather than echo delay *per se*". Therefore, both target range and echo-acoustic flow determine the overall response in this cortical area. Bartenstein et al. (2014) suggest that the flow-induced modifications could serve to elicit adjustments of echolocation and flight behaviour, as we observed them in our experiments. They proposed a simple threshold mechanism that triggers adaptive motor behaviours, whenever the population activity in the target range map exceeds a certain value. When the bats induce a path correction towards the side eliciting weaker echo-acoustic flow, distance to this wall automatically decreases. This might in turn increase the representation of target range towards this lateral structure, which formerly elicited negligibly weak echo-acoustic flow. So far, it is unclear how the presence of (bilaterally) flanking structures influences the cortical target range map, especially when these elicit echo-acoustic flow of different strength, or when the lateral distance to either of them is changed. Other studies that aimed to investigate how the presence of multiple targets changes the response characteristics of echo delay sensitive neurons in the cortical map for target range found that a meaningful stimulus history, representing a flight path towards and over a series of objects, sharpens the tuning in these neurons (Beetz et al., 2016b) and that the neurons tend to respond most selectively to the closest target, while a response to targets at a distance is suppressed (Beetz et al., 2016a). Greiter and Firzlaff (2017) simulated multiple objects not beneath, but lateral to flight paths. They found that in a population of neurons in the cortical target delay map, individual neurons selectively locked to one of two presented targets. Greiter and Firzlaff (2017) showed that individual neurons selective for the closest target are active early on, but that even before the first target was passed, neurons selective to the subsequent target started to respond, hinting towards a sequential processing of multiple targets. The authors checked if this behaviour could be ascribed to changes in a single parameter like e.g. echo delay or echo intensity. However, this was not the case and the authors conclude that their results are due to complex interactions between a variety of parameters, as elicited by echo-acoustic flow.

It is conceivable that the different parameters producing echo-acoustic flow, like target range and angular velocity, are competing factors that contribute to the cortical representation of target range. In order to test this, new electrophysiological and psychophysical experiments should be carried out. An apt paradigm to test this would include a corridor with adjustable width. The side walls could consist of reflectors with adjustable translational spacing (determining the strength of echo-acoustic flow). A schematic of this simulated scenario is shown in Fig 4.1. By presenting scaled versions of a corridor (blue and green structures in the picture) to a bat flying at constant speed, one could keep the angular velocity (and the target strength) constant while changing the target distance.

Of course, a physical implementation of this paradigm would be extremely tedious. A more elegant realization could be achieved using virtual object technology (Schmidt, 1988) and a wind tunnel. Virtual object technology exploits the fact that the acoustical properties of a scene can be captured by its impulse response, also referred to as the acoustical image. An impulse response comprises the reflection characteristics of an object or a scene to an acoustic impulse.

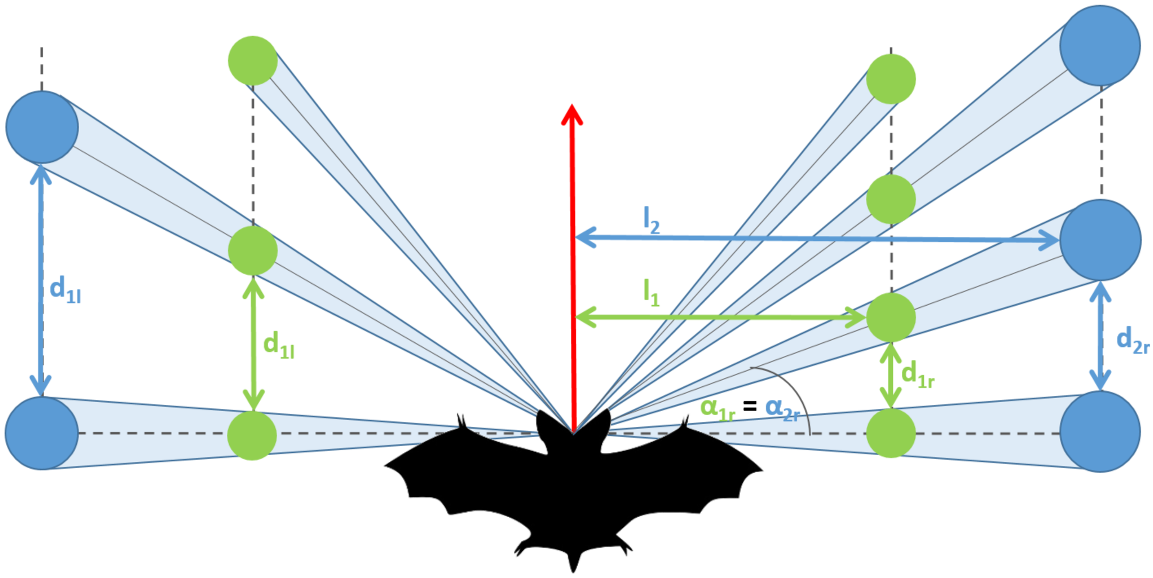

Figure 4.1: Simulated scenario that can be used to test which cues bats rely on for flight guidance in a corridor lined by multiple lateral reflectors. Cues that should be tested include echo-acoustic flow, target strength, lateral reflector distance (or echo-delay). Echo-acoustic flow is mediated by covariant changes in different parameters, whose relative importance can be assessed individually by altering the translational spacing between the laterally placed reflectors (parameter d), corridor width (parameter l times 2) and changes in sonar aperture (determined by the radius of the reflectors, light blue color-coded angles in the figure).

An acoustic impulse is a theoretically infinitely short signal with infinite amplitude, comprising all excitation frequencies with the same amplitude. Virtual objects are created by convolving the bat's echolocation call with the impulse response (of the object or scene to be simulated). The result is played back as the echo of the virtual object. An unquestionable advantage of virtual object technology is that it allows to flexibly change the spatial layout. This technology would allow for a randomized presentation of differently scaled flight corridors. Flight path adjustments with respect to midline would allow assessing the impact of different parameters that might contribute to flight guidance, independently. Target delay encodes for target range and is therefore crucial to avoid collisions. The angular velocity can be used to determine passage speed and might hence be used to regulate not only the flight position relative to the side walls, but also flight speed. Target strength co-varies with the area of a reflective surface and was speculated to be the main cue bats use to estimate object size (Simmons and Vernon, 1971). However, previous experiments have shown that the sonar aperture, the spread of angles of incidence that impinge on the bat's ear, plays an important role for bats when assessing object width (Heinrich et al., 2011). This parameter naturally changes when objects approach and pass by. The changes in width/sonar aperture of an approaching object could thus contribute to auditory looming perception and the perception of echo-acoustic flow. Obstacle avoidance experiments with naïve bats have shown that the animals avoid small (real) targets, but that they do not show an increased evasive response, when target strength of a virtual object played back from a single speaker was increased (Goerlitz et al., 2012). The authors discuss that the bat not perceiving the virtual object as a spatially extended structure (despite the increased target strength) probably causes this lack of increase in evasive flight response. A single speaker can only simulate a rather small aperture that corresponds to a thin, point-shaped object. In order to create stimuli that evoke the perception of an extended object, the virtual object must be simulated by an array of neighbouring speakers. Such an array in combination with a wind tunnel (similar to the experiment outlined in Fig 4.1) would allow to test the effect of naturally occurring expansion (in terms of sonar aperture) of single or flanking approaching objects on bat flight guidance.

The same paradigm and stimulation could be used to assess the effects of these parameters on the cortical map for target range. Optimally, one would record telemetrically with an electrode array from posterior dorsal field of the auditory cortex of a behaving bat while it is performing the psychophysical experiment in the wind tunnel. This would allow correlating activity patterns in the cortical map for target range to flow-specific flight path adjustments.

4.3 Echo-acoustic scanning via noseleaf and ear movements in phyllostomid bats

4.3.1 Appearance of call-correlated noseleaf and ear movements

In the second set of experiments that was conducted for this thesis we aimed to investigate how phyllostomid bats employ facial features of their echolocation system for the analysis of echo-acoustic flow. To this end, we monitored movements of the noseleaf and the ears as well as the general echolocation behaviour while the bat was moved along or towards different reflectors. We found movements of both the noseleaf and the outer ears that were correlated with the emission of echolocation calls. This experiment shed light on the echo-acoustic sampling of the surroundings in phyllostomid bats. However, how bats employ or alter this behaviour in ecologically relevant situations (like prey interception or obstacle avoidance) remains an open question. Because animals fly in a restricted three-dimensional volume in a wind tunnel, it would be possible to conduct experiments on the movement of the facial features of the echolocation system, like presented in the second chapter of this thesis, during free flight. A reconstruction of a structure is possible as long as the entire volume in which the structure is moving can be covered by at least two perspectives simultaneously. In our experiment, we installed a camera acquiring two perspectives of the bat via mirrors on the cart that also carried the bat. This enabled us at each point in time (and at each place the bat passed) to acquire a stereo-image of the bat. In a wind tunnel, the volume in which the bat flies would be bigger compared to the very restricted volume in which the animal can move in our setup. However, in the wind tunnel one does not face the challenge of installing a multiple-perspective system on a small cart. Instead, multiple cameras or motion capture systems could be mounted in various locations and would allow for a simultaneous acquisition of at least the two required perspectives. This would allow monitoring how our bats employ the accessory features of their echolocation system in free flight during tasks like obstacle avoidance or passages through corridors with different dimensions, in the same setup as suggested previously (see Fig. 4.1). Probably such an experiment would reflect a more natural situation: In our experiments, the bats were moved passively and therefore could not influence echo-acoustic flow by compensational flight manoeuvres. That is, the perceived echo-acoustic flow was solely determined by the (externally controlled) relative motion of the animals with regard to the stimuli plus the echo-acoustic sampling strategy. In free flight, however, the animal can alter the experienced echo-acoustic flow additionally by compensational flight manoeuvers, which will most certainly alter the way the animal samples its surroundings.

4.3.2 Function of call-correlated noseleaf and ear movements

Two more questions that we could not completely answer using our setup are (1) how the observed movements of the noseleaf translate into the emission characteristics of the echolocation call and (2) how the observed ear movements alter echo perception. As discussed in Kugler and Wiegrebe (2017), previous results and our observations hint at an involvement of noseleaf movements in elevational beam steering. Our setup contained only a horizontal microphone array that allowed us to monitor azimuthal beam orientation, but lacked a vertical microphone array which would have obstructed the volume in front of the bat. However, in order to assess the influence of the observed bending and stretching noseleaf movements on beam orientation, a two-dimensional microphone array is required. Future experiments should target how the call-correlated bending and stretching movements of the noseleaf translate into changes in beam orientation and beam focus. For such an approach, animals will need to be head-fixed to exclude an influence of head movements. Echolocation will need to be elicited, either by simulated self-motion of the animal, like in our experiment, or by moving a physical stimulus towards the bat, like in previous studies that found range-specific adjustments in echolocation behaviour in such tasks (Aytekin et al., 2010; Linnenschmidt and Wiegrebe, 2016; Wohlgemuth et al., 2016a). Such an approach would allow assessing, if the range-specific changes in echolocation behaviour are accompanied by changes in sampling behaviour involving the facial features of the echolocation system.

As discussed in chapter 3 of this thesis, the observed call-correlated ear movements might serve to adjust the focus of the receiving system. The raised ear position indicates that the receiving system is directed towards the front for echo reception. This frontally oriented focus is in line with the directionality of the emission pattern and the directionality of the receiving system of *Phyllostomus discolor* (Vanderelst et al., 2010; Firzlaff and Schuller, 2003). An electrophysiological study revealed that the auditory cortex of *Phyllostomus discolor* contains a population of neurons with focused, forward-oriented receptive fields, indicating that the directionality of these neurons is matched to the frontward-oriented directionality of the emitting and the receiving echolocation system of these bats (Hoffmann et al., 2010).

To investigate how ear movements alter the echo before it impinges on the bat's tympanum, pinna shape and orientation needs to be determined in 3D. Using 3D shape models, the spectrum of a signal at the tympanum can be predicted. This method was successfully applied to predict how ear shape and orientation of rhinolophid bats influences the directionality of their receiving system (Gao et al., 2011).

4.4 Studying echo-acoustic flow as perceived in behaving bats

In order to understand which parameters trigger flight path adjustments towards an area where the bat experiences weaker echo-acoustic flow, it would be necessary to measure the signal as perceived by the animal. This, however, is extremely complicated, if not impossible, to do in a behaving animal without impairing the natural behaviour. Previous studies have measured how stimuli are altered between the place of origin and the bat's tympanum, depending on the relative position to the animal (see paragraph on HRTFs in section 1.3.1). For this kind of measurements, small microphones are inserted into the ear canals of a sacrificed specimen or a model head of a bat. The microphones are placed at the location where the tympanum would be situated via holes in the animal's skull. This allows measuring how a signal is altered when impinging on a bat's ear from a defined direction in space (Aytekin et al., 2004; De Mey et al., 2008; Firzlaff and Schuller, 2003; Obrist et al., 1993). In cats it was confirmed, that not only the relative location of signal origin, but also the posture of the animal's ear determines how the signal is modified (Young et al., 1996), stressing that both ear orientation and deformation alter the perception of sound, and consequently the perception of (echo-)acoustic flow. Data from sacrificed specimens or bat dummies can therefore not be used to investigate how the dynamic features introduced by the ear, head or even body movements of a flying bat influence what the animal perceives. More recent studies aimed to investigate the stimuli impinging on a navigating bat by mounting a small device comprising a microphone onto the bat (Cvikel et al., 2015; Hiryu et al., 2005; Stidsholt et al., 2017). Using this approach one can measure echoes to echolocation calls produced by the navigating animal itself during natural flight. These measurements likely capture the modifications introduced by an animal's body movements, because the microphone is usually mounted on the animal's back and is hence aligned with its body axis. However, both static modifications introduced by the ear morphology as well as dynamically changing modifications introduced by ear or head movements cannot be measured using this method. To achieve this, it would be necessary to record the signal at the tympanum of a navigating animal without obstructing both the natural behaviour of the animal and the perception of the signal. This, however, is technically hard to achieve, because a microphone at the place of the bat's tympanum will alter the propagation of the signal to the middle and inner ear, i.e. it will alter the way the animal perceives the signal. Consequently, the animals would change their flight behaviour, which renders the recording of naturally occurring echo streams impossible. Another method using a drone equipped with ultrasonic speakers and microphones likewise aims to record the acoustic scene as experienced by the navigating bat.

A drone can be used to measure how echoes to playbacks from echolocation calls are reflected back to different locations. To model echo perception during natural flights, the locations where echolocation calls were sent out by bats behaving in a psychophysical experiments can be used to recreate the echo streams received by the behaving animals retrospectively (e.g. Warnecke and Moss 2016). Similar techniques using fish dummies with water flow sensors are used in limnological and marine research, to assess to which forces and water flows fish are exposed at different regions in the water (e.g. Tuhtan et al. 2017). But again, the drone technique bares the same drawbacks as recordings with a microphone mounted onto the animal's back: static modifications introduced by the ear morphology as well as dynamically changing modifications introduced by ear and head movements cannot be incorporated using this method. To overcome this problem, head and ear movements as well as the 3D shape of the ears would have to be recorded during natural flights. Such knowledge can be used to model the echoes that arrive at the bat's tympanum (Gao et al., 2011). Finally, a sound is further modified by the animal's auditory system. Filtering in the sensory periphery and neural integration determines the internal representation of a physical stimulus (see section 1.1). As a consequence, the internal representation of an auditory stimulus differs spectro-temporally from the stimulus that impinges on the animal's ear. Auditory models can be used to model how sound is filtered in the periphery auditory system (e.g. Wiegrebe 2008). The neural representation of the stimulus is the substrate from which the bat can extract information. For a comprehensive understanding of how a bat perceives echo-acoustic flow, future studies should therefore aim to understand how the surroundings of a flying bat are internally represented. This requires various steps that can in part be answered by a combination of the methods mentioned above. Data on the flight behaviour of a navigating bat is required to determine where (with respect to surrounding objects) and when the animal emits echolocation calls. The echolocation calls and the characteristics of the outgoing echolocation beam (directionality and steering) need to be incorporated to capture echo-acoustic adjustments to an acoustic scene and to compute all echoes returning to the bat (to each echolocation call). Furthermore, information on the shape and the relative orientation of the ears of the bat at the time of echo reception are required to compute how each echo is modified in the outer ear. Finally, an auditory model is required to predict the internal representation of the echoes, i.e. the basis for the animal's reactions.

4.5 Gaze stabilization and gaze strategies during flight

When an animal moves quickly through a complex environment, the resulting sensory flow can be very high. For fast angular motion between the environment and the sensor, sensory flow might potentially be too high for the sensory system to spatially resolve information in detail. Fast angular motion likely occurs when an observer and an object in close proximity move towards each other (in the form of a translational movement), or when the sensor is moved by a rotational movement, e.g. by a head turn. In the visual system, such fast motion can produce so-called motion blur and it can affect the perception of the three-dimensional environment (Srinivasan and Bernard, 1975; Yarbus, 1967). To cope with this challenge, visually oriented animals have evolved different strategies that aim for gaze stabilization. Eye movements that counterbalance the relative motion of the object are one possibility to lock the gaze onto a moving target, until the eyes make a rapid movement, a *saccade*, towards the next point of fixation (Yarbus, 1967). This strategy allows maximizing the time a stable percept can be obtained while minimizing the time where motion blur occurs. However, this strategy can only be applied by animals with motile eyes. In some animals eyes and head are fused. For example the lenses of insect eyes are rigidly connected to the animal's head. Even though the retina can to some degree be displaced along the focal plane, causing small shifts of the optical axes along the focal plane, the animals can move the head in yaw, pitch and roll relative to the thorax, allowing to turn the head voluntarily during flight manoeuvers or to stabilize the head when the thorax is involuntarily turned by turbulences (Hengstenberg, 1992). It was found that these head-eye movements are also employed for gaze stabilization during flight (Schilstra and van Hateren, 1998): in blowflies, thorax movements producing yaw turns (thorax saccades) are accompanied by later and quicker movements of the head, that minimize the time in which motion blur occurs due to a gaze shift. Hateren and Schilstra (1999) argue that this behaviour serves to separate the optic flow into its rotational component (that occurs during thorax saccades) and its translational component (occurring in between thorax saccades). Rotational optic flow induces rigid rotations of the entire optic array and depends solely on the rotational movement, but not on the spatial arrangement of the surroundings. It therefore cannot be used to extract depth cues that are needed e.g. for obstacle avoidance (Gibson, 1950; Koenderink, 1986). Translational optic flow depends on the distance of objects in the surroundings, and it is hence stronger for near objects and weaker for distant objects (Gibson, 1950; Koenderink, 1986). Accordingly, translational optic flow can be used to extract depth information. This implies that these head movements might be beneficial for blowflies because they (1) reduce motion blur and (2) minimize the time they are exposed to rotational optic flow where they cannot obtain depth information about their surroundings (Hateren and Schilstra, 1999; Schilstra and Hateren, 1999; Schilstra and van Hateren, 1998). A study in zebra finches revealed that birds use a similar technique, separating phases of rotational and translational optic flow. They do this by applying fast head saccades to shift the gaze, while stabilizing gaze in between saccades (Eckmeier et al., 2008). This finding was further confirmed by studies in rapidly manoeuvring lovebirds (Kress et al., 2015). Many bat species have motile ears. Our data suggest that phyllostomid bats point their motile ears towards the front for echo reception, parallel to the head axis. This indicates that they use the ears to regulate the focus of the receiving echo-acoustic system, instead of producing ear saccades for target tracking. In future experiments one should address the question, if and how bats stabilize their echo-acoustic gaze. Similar to insects and birds, this could be achieved by head saccades. However, beyond gaze stabilization, this technique serves to extract depth information. Bats have direct information on the distance of close-by objects via echo delay.

A separation of rotational and translational echo-acoustic flow might thus be less important in acoustically guided bats than in visually guided flyers.

4.6 Echo-acoustic sampling in other species

Nowadays, autonomous vehicles and autonomously navigating technical devices become more and more important. Therefore, it might be of special interest to investigate the echolocation systems and the respective sampling strategy in various echolocating species. This might help to identify common principles that could be used for the design of biomimetic sensors. To understand if analogous sampling strategies have evolved in different echolocation systems, one should target sampling, especially beam steering strategies in other (skilled) echolocators. One example is echo-acoustic sampling in orally emitting bats. We know that orally emitting bats adjust the mouth gape and thus the emitter aperture to increase or decrease the directionality of the emitted call (Kounitsky et al., 2015). Observations show that pipistrelle bats additionally use beam scanning to sample their surroundings (Seibert et al., 2013). However, it remains to be uncovered, if they have a mechanism to steer their beam independent of head movements.
Investigating beam steering behaviour in echolocating species that lack (motile) outer ears, which could further be recruited to scan the surrounding might be especially interesting. For those species, it might be even more important to produce a directional call, because they lack a part of the dynamic directionality of the receiving system. Odontocetes are promising candidates for such a study. In these animals, sound production works different than in bats: sound is produced not via the larynx, but via the so-called phonic lips, present in two pairs and situated in nasal complex in the blowhole. The sound is transmitted through the prominent *melon*, which sits on top of the phonic lips. The melon is high in fat content and is coupled to at least one pair of phonic lips (Au et al., 1986; Cranford et al., 1996). Therefore, it is conceivable that the melon has a function in transmitting sound into the water. It was suggested that the melon acts like an acoustic lens (Huggenberger et al., 2009). Odontocetes can alter beam orientation to sample objects at different directions, and surprisingly, without using head movements (Ladegaard et al., 2017; Madsen et al., 2010; Moore et al., 2008). As the melon sits on top of massively innervated muscles (Huggenberger et al., 2009), it is conceivable that the melon might be involved in beam focusing and steering (both in azimuth and elevation).

Flow fields convey information that is crucial for navigation. They are an important cue for the guidance of movements in a variety of animals. Visually guided animals use optic flow for flight guidance and to adjust flight velocity. Our results reveal that bats, as acoustically guided animals, use echo-acoustic flow for flight guidance, but not to adjust flight velocity. Sensory flow is hence a common principle for flight guidance, independent of the fundamentally different peripheral representation of flow across vision and echolocation.

The weighting of flow information compared with other cues might, however, be different in different sensory modalities. To extract meaningful flow information, animals must be able to adjust their sensory system in a way that allows them to spatially and temporally resolve changes in the environment. In the visual system this is accomplished by alternating between periods of gaze stabilisation and fast changes in gaze direction (via saccades). The direction and focus of gaze is adjusted to the respective point of fixation. Our results suggest that phyllostomus bats can adjust the focus of their echolocation system via call-correlated ear movements. The direction of the echo-acoustic gaze is likely determined by a combination of head direction and beam direction, mediating the azimuthal and elevational components of the echo-acoustic gaze.

Recent research, including this thesis, suggests that the dynamics of spatio-temporally plausible stimulation, as occurring in ecologically relevant contexts, can significantly affect and alter the behavior of animals. Classically, spatial representations of stimuli have been assessed in terms of randomly sampled spatial tuning curves, i.e., the dynamic scene has been discretised and the perceptual and neural representations of dynamic scenes have be thought to derive from a sequence of such static images. Recent research has shown, however, that spatially dynamic scenes are rarely predicted correctly from concatenating static images. As such, the current flow-field stimulation highlights the need of neuroethological research for realistic, spatio-temporally dynamic stimulation to understand how animals assess complex spatial layouts through the combination of spatial sensation and self motion.

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Declaration of contribution as co-author

Echo-acoustic flow affects flight in bats
by Kathrin Kugler, Wolfgang Greiter, Harald Luksch, Uwe Firzlaff and Lutz Wiegrebe.

Kathrin Kugler, Uwe Firzlaff and Lutz Wiegrebe designed the experiments and wrote the application for the animal experiment; Kathrin Kugler and Wolfgang Greiter built the setup; Kathrin Kugler wrote the experimental programs, executed the experiments and analysed the data; Kathrin Kugler, Wolfgang Greiter, Harald Luksch, Uwe Firzlaff and Lutz Wiegrebe wrote the paper.

Echo-acoustic scanning with noseleaf and ears in phyllostomid bats
by Kathrin Kugler, and Lutz Wiegrebe.

Lutz Wiegrebe and Kathrin Kugler designed the experiments, wrote the application for the animal licence and wrote the paper. Lutz Wiegrebe, Kathrin Kugler, Florian Sojak and Stefan Dietrich built the setup. Kathrin Kugler wrote the experimental programs, acquired the data, and did the analyses. Christiane Bühning and Lilian Diem My Levinh helped with data collection and data analysis in their Master and Bachelor thesis (under the supervision of Kathrin Kugler), respectively. Christiane Bühning also contributed to the development of the labeling technique and the video analysis. Daria Genzel did comprehensive groundwork on labeling and tracking of facial features of bats, which was partly incorporated in the methodology of this work.

(Lutz Wiegrebe)

(Kathrin Kugler)

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Education

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IN PRESS

- **Kugler K, Wiegrebe L.** *Echo-acoustic scanning with nose leaf and ears in phyllostomid bats.* J Exp Biol. in press 2017

PUBLISHED

- **Kugler K, Greiter W, Luksch H, Firzlaff U, Wiegrebe L.** *Echo-acoustic flow affects flight in bats.* J Exp Biol. 2016 Apr 4. pii: jeb.139345. [Epub ahead of print] PubMed PMID: 27045094.
- **Kugler K, Wiegrebe L, Gürkov R, Krause E, Drexl M.** *Concurrent Acoustic Activation of the Medial Olivocochlear System Modifies the After-Effects of Intense Low-Frequency Sound on the Human Inner Ear.* J Assoc Res Otolaryngol. 2015 Dec;16(6):713-25. doi: 10.1007/s10162-015-0538-4. Epub 2015 Aug 12. PubMed PMID: 26264256; PubMed Central PMCID: PMC4636592.
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Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

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